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ADVANCES IN THE USE OF PRODRUGS FOR DRUG DELIVERY TO THE EYE

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Abstract

Introduction: Ocular drug delivery is presented with many challenges, taking into account the distinctive structure of the eye. The prodrug approach has been, and is being, employed to overcome such barriers for some drug molecules, utilizing a chemical modification approach rather than a formulation based approach. A prodrug strategy involves modification of the active moiety into various derivatives in a fashion that imparts some advantage, such as membrane permeability, site specificity, transporter targeting and improved aqueous solubility, over the parent compound.

Areas covered: The following review is a comprehensive summary of various novel methodologies and strategies reported over the past few years in the area of ocular drug delivery. Some of the strategies discussed involve polymer and lipid conjugation with the drug moiety to impart hydrophilicity or lipophilicity, or to target nutrient transporters by conjugation with transporter specific moieties and retrometabolic drug design.

Expert opinion: The application of prodrug strategies provides an option for enhancing drug penetration into the ocular tissues, and overall ocular bioavailability, with minimum disruption of the ocular diffusion barriers. Although success of the prodrug strategy is contingent on various factors, such as the chemical structure of the parent molecule, aqueous solubility and solution stability, capacity of targeted transporters and bioreversion characteristics, this approach has been successfully utilized, commercially and therapeutically, in several cases.

Keywords

Lipid prodrug; Polymer-conjugated prodrug; Retrometabolic drug design; Transporter targeted drug delivery

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1. Introduction

1.1. Anatomical and physiological considerations for drug delivery

Ocular drug delivery remains a major challenge for formulators because of the unique structural organization of the eye.¹⁻⁴ To understand the fate of the drug in the ocular tissues and overcome the barriers to drug absorption, it is necessary to study the anatomy of the eye (Figure 1), which is organized into an anterior segment and a posterior segment.⁵ Therapeutic agents may need to be targeted to one or both segments of the eye. The anterior segment is composed of the crystalline lens suspended from the ciliary body, and the structures anterior to it; namely, the cornea, the iris and the two chambers containing aqueous humor: the anterior and the posterior chambers. The anterior chamber contains approximately 0.25 mL of aqueous humor and is bound anteriorly by the back of the cornea and posteriorly by the iris and a part of the ciliary body. The posterior chamber consists of approximately 0.06 mL of aqueous humor and is bound anteriorly by the iris and a part of the ciliary body and posteriorly by the crystalline lens.^{6, 7} The aqueous humor is a clear, colorless fluid, secreted by non-pigmented epithelial cells of the ciliary body, with a chemical composition similar to that of blood plasma, but with a low protein content.⁸ The sclera, retina-choroid, vitreous humor and optic nerve make up the posterior segment, of which the vitreous humor, a hydrophilic gel matrix, makes up 80% of the volume of the eye.^{1, 6, 9-12}

Topical delivery is the preferred and patient-friendly technique for treating diseases of the anterior segment, and involves instillation of the eye drops into the conjunctival cul-de-sac. A topically applied formulation, however, has to overcome multiple pre-corneal barrier mechanisms, such as dilution, overflow, tear-fluid enabled lacrimal drainage and conjunctival absorption resulting in elimination from the pre-corneal area.^{5, 13, 14} The ocular tissues maintain a highly regulated environment for visual cells and transparent tissues. Ocular barriers, namely the Blood-Aqueous Barrier (BAB) and the Blood-Retinal Barrier (BRB), play a vital role in the protection of the eye and the maintenance of ocular functions by restricting the entry of xenobiotics, consequently challenging the passage of therapeutic drug molecules into the ocular tissues.¹⁵ The multiple biological barriers to ocular drug delivery are illustrated in Figure 1.

The cornea is the outermost tissue in the anterior ocular segment and consists of five layers: the epithelium, Bowman's membrane, the stroma, Descemet's membrane, and the endothelium.^{16, 17} The corneal epithelial cells are connected to each other via desmosomes and express tight-junctions that act as a rate-limiting barrier for hydrophilic molecules.^{18, 19} Lipophilic drugs, depending on biopharmaceutical characteristics such as solubility, partition coefficient, ionization and charge and polar surface area, demonstrate better transcellular permeation compared to hydrophilic molecules.^{20, 21} The stroma underlying the corneal epithelium, however, is hydrophilic in nature.²² Additionally, efflux pumps present on the corneal and conjunctival membranes restrict the entry of substrates (mostly lipophilic in nature) into the deeper ocular tissues.^{17, 23-25} Thus, therapeutic agents must possess optimal physicochemical and biopharmaceutical characteristics to permeate efficiently across the total corneal membrane. The non-corneal route of absorption encompasses conjunctival

pathways, and favors hydrophilic and large polar molecules.^{1, 26, 27} The BAB and the BRB also limit the passage of both hydrophilic and lipophilic molecules by virtue of the tight-junctions and efflux mechanisms present.^{1, 24, 28-31} Both systemic and periocular delivery are affected by the presence of the BAB and BRB.

The following sections will briefly discuss some of the approaches overcoming the challenges in ocular drug delivery, followed by an in-depth review of the prodrug approach.

1.2. Formulation-based strategies for ocular delivery

Retention of the formulation on the ocular surface and penetration of the active ingredient into the ocular tissues are critical parameters governing the effectiveness of topical application. Conventional formulation strategies involve the use of viscosity enhancers such as hydroxyethyl cellulose and hydroxypropyl methyl cellulose, or penetration enhancers such as cyclodextrins, benzalkonium chloride or surfactants, in solution and suspension formulations.³² The formulator may also opt for gels, ointments and other viscous formulations to improve residence time on the surface.^{33, 34}

Improving the viscosity improves the contact time of the active ingredient with the corneal surface and improves flux but does not improve the corneal membrane permeation characteristics of the molecules. Nanocarriers, such as liposomes, niosomes, lipid nanoparticles and nanoemulsions, are a few of the innovative strategies that can encapsulate a wide range of drugs. Vesicular carriers such as liposomes have an added advantage in that they can load both hydrophilic as well as lipophilic molecules and also provide an option for surface modification. The use of a vesicular system is, however, associated with problems such as limited drug loading, short-term stability, unwanted side effects and difficulty with sterilization.^{35, 36} Lipid-based nanoparticulate systems have recently gained interest for ocular delivery. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) show promise because of better biocompatibility, enhanced corneal retention and permeation of the nanoparticles.^{37, 38} The formulation approaches, however, do not change the ability of the compounds themselves to diffuse across membranes.

1.3. Prodrug derivatization for ocular delivery

Chemical derivatization strategies such as prodrug derivatization, in contrast, have been employed to improve trans-membrane permeation. The prodrug approach provides a strategy to modulate the lipophilicity, solubility, ionization and stability of the drug candidate and, thus, improve ocular penetration.³⁹ The prodrug concept was introduced to the field of ophthalmology in 1976 by Hussain et al.⁴⁰ to enhance the absorption of a highly polar molecule, epinephrine, through lipid membranes. Since then, various prodrugs have been designed to improve physicochemical properties of therapeutic agents. Currently, a few of the prostaglandin prodrugs used to treat ocular hypertension due to open angle glaucoma are commercially available. These include latanoprost, approved in 1996³³; travoprost, approved in 2006; bimatoprost, approved in 2010; and tafluprost, approved in 2012. Slight structural differences exist between these analogs. For example, the carbon-1 position of the alpha chain of latanoprost or travoprost has a hydrolysable isopropyl ester group that is converted into its biologically free acid by ocular esterases. Unlike latanoprost and travoprost,

bimatoprost has an ethyl amide group in the carbon-1 position and has been categorized as a prostamide, a relatively recently described class of agents that is said to activate novel receptors.³⁴

Nepafenac (2-amino-3-benzoylbenzeneacetamide), an amide prodrug of amfenac, an arylacetic acid derivative, is another example of an ophthalmic prodrug formulation on the market.^{41, 42} Nepafenac ophthalmic suspensions (0.1% and 0.3%) have been approved for the treatment of pain and inflammation associated with cataract surgery.

In view of these success stories and the potential benefits of this strategy, it is not surprising that a significant amount of research continues in the area of prodrug design to improve ocular interventions. Some of the developments in this area are reviewed herein.

2. Polymeric prodrug systems

The use of functionalized polymer-drug conjugates as a stimuli sensitive and targeted approach has been used extensively for oral delivery.⁴³ Polymer conjugation would alter the pharmacokinetics and distribution of the drug molecule. The safety and efficacy of the formulation would depend on the rate of release of the drug from its polymeric linkage. The drug-polymer conjugate should be stable enough to remain inactive until it reaches the site of action and should then release the drug. Premature release of the drug molecule may result in unwanted toxicity, affecting the safety profile of the drug. However, upon reaching the desired target destination, release of the drug is then required to achieve efficacy. Thus, there arises a need for a balance between conjugate stability and bioreversion rate.^{44, 45}

2.1. PEG-based prodrugs

One of the simplest polymers that can be used for drug conjugation is polyethylene glycol (PEG). High molecular weight PEG has been reported to improve the plasma half-life of small molecules in mice.⁴⁶ PEG-protein conjugates are gaining attention because of the ability of PEG to protect against proteolytic degradation.^{44, 47} Conjugation of PEG to proteins is reported to mask the protein surface and inhibit antigen-processing cells. The use of PEG in ophthalmics can improve the hydrophilic-lipophilic balance, enhancing transcorneal and transcleral penetration. Foroutan et al.⁴⁸ studied the hydrolysis of PEG esters of hydrocortisone 21-succinate (H-PEGs) in commercial esterases and the bovine cornea. The authors reported that the use of H-PEG400 increased the rate at which hydrocortisone diffused through excised bovine cornea, compared to hydrocortisone alone, and also that an increase in the polymer chain length resulted in a significant decrease in enzymatic hydrolysis.⁴⁸

2.2. Molecular hydrogels / supramolecular nanofibers

A number of small molecules are known to self-assemble into fibrillar networks to form gels in the presence of various solvents and are known as low-molecular weight gelators. In an aqueous solution, these gelators help form macroscopic supramolecular hydrogels. Because they do not involve the use of any excipient, they are considered to be biocompatible and biodegradable.⁴⁹⁻⁵¹ Molecular hydrogels are carrier-free drug delivery systems, formed by the self-assembly of pro-moieties of small molecules. It has been stated, 'Nanofabrication is

self-assembly at a molecular level driven because of chemical complementarities and shape.^{52, 53} This strategy has been utilized in the past for enzyme-triggered degradation.^{54, 55} Novel prodrug systems using hydrogelators, such as prodrugs of acetaminophen, have been used to form self-assembling hydrogels, which have the capacity to encapsulate another drug; for example, curcumin. Upon enzymatic degradation, this system released both the drugs at physiologically simulated conditions *in-vitro*.⁵⁴

Li et al.⁵⁵ synthesized supramolecular nanofibers of Triamcinolone Acetonide (TA), a corticosteroid used in the treatment of uveitis and ocular inflammation, via the ester bond hydrolysis of its prodrug, Succinated Triamcinolone Acetonide (STA). STA was synthesized from TA by a one-step reaction. The hydrogel formed upon the dissolution of the STA in phosphate buffer solution (pH = 7.4) and maintenance for approximately 35 h at 37°C (Figure 2). The hydrogel exhibited 42% release in 12 h in comparison to the 15% release shown by the suspension, and better transcleral penetration in comparison to the marketed TA suspension. The authors attributed the rapid release of TA from the hydrogel to its weak nature and noted that polymer additives could be added to strengthen the hydrogel to prolong drug release. However, a retrobulbar injection of the suspension showed better therapeutic effect than the STA hydrogel in an experimental autoimmune uveitis model in Lewis rats, as the suspension formed a depot of microparticles, releasing the drug over a prolonged period of time.^{55, 56}

2.3. Lactic acid-based polymers

In a similar approach, Veurink et al.⁵⁸ designed lactic acid-based co-polymer and prodrug systems to sustain the release of the vasodilator L-lactate that can be used in conditions involving the obstruction of retinal vasculature that can lead to various diseases of the posterior segment. An intravitreal injection of L-lactate has a limited duration of action because it is a small molecule and is eliminated quickly from the eye.^{57, 58} The goal of the investigators was to design a prodrug system that released L-lactate over a period of weeks. Poly-lactic acid on its own resulted in the formation of a slowly degrading, non-injectable solid, indicating the need for incorporation of plasticizers in the system. Thus, a biodegradable co-polymer system, poly (L-lactic acid-co-L, D-2-hydroxyoctanoic acid) was designed as an intravitreal injectable depot system, the release of which, as extrapolated from the data obtained by Brazitikos et al.⁵⁹, is expected to sustain a therapeutic effect for approximately 14 days. The co-polymer showed good *ex vivo* biocompatibility with retinal tissues. Comparison of the release of L-lactic acid in vitreous humor (*ex vivo*) and phosphate buffer (*in-vitro*) revealed a similar release profile in both release media, suggesting that the enzymes in the vitreous humor did not substantially influence the degradation rate of the co-polymer and that the release of L-lactic acid would not be significantly affected by inter-patient variability in enzyme levels and activity.⁵⁸

3. Pro-pro drugs/ double prodrugs

Cascade latentiation or the pro-prodrug approach may be utilized to impart high stability as well as bioreversibility. Prodrugs that undergo non-enzymatic (chemical) hydrolysis to release the active drug face stability issues. This problem may be controlled by utilizing the

double-prodrug or the pro-prodrug strategy, derivatizing the prodrug for a site-specific enzymatic hydrolysis, which would then lead to spontaneous release of the parent drug at the target site and an inactive prodrug at other sites.⁶⁰

Cyclosporine A, an immunosuppressant used in the treatment of dry eye syndrome, is currently available as an FDA approved emulsion formulation.⁶¹ Lallemant et al.⁶² studied a prodrug approach for improving the water-solubility of Cyclosporine A by esterification of the free hydroxyl residue in position 1 (UNIL088). The solubility of UNIL088 in isotonic phosphate buffer solution (pH 7) was approximately 25,000 times higher than that of Cyclosporine A.⁶² The drug was released after the dipeptide sarcosine-serine-(acyloxy) alkyl-oxy-carbonyl chain, carrying a phosphate group as a solubilizing moiety, was removed. These molecules have to undergo chemical as well as enzymatic hydrolysis and, thus, can be categorized as pro-prodrugs.⁶³ The enzymatic and chemical conversion of UNIL088 was studied in rabbit tears at physiological temperature. The primary biotransformation pathway involved hydrolysis of the terminal ester of the (acyloxy) alkyl-oxy-carbonyl group followed by a cascade of hydrolysis reactions to form the intermediate, pSer-Sar-CsA. Cyclosporine A was then released by esterase-like activity by the tear fluid (Figure 3).⁶²⁻⁶⁶

Rodriguez-Aller et al.⁶⁸ compared the ocular tolerance and *in-vivo* ocular distribution of the water soluble pro-prodrug-solution (UNIL088, also known as OPPH 088) and that of the marketed CsA oil-in-water emulsion. The corneal surface injury evaluated after the administration of the pro-prodrug was approximately 5% of the observed area, whereas for the marketed emulsion, it was approximately 18% of the observed area. The ocular distribution studies suggested that the pro-prodrug achieved deeper ocular penetration, with Cyclosporine A concentrations between 50 and 300 ng/g of tissue in cornea, conjunctiva, iris-ciliary bodies and retina, higher than the Cyclosporine A levels obtained with the emulsion.⁶⁸ The authors concluded that the pro-prodrug approach is a safe and efficient approach for the treatment of dry eye syndrome because of lower elimination and higher availability of Cyclosporine A, especially in the conjunctiva, in comparison to that from the emulsion formulation.⁶⁸⁻⁷⁰

4. Retrometabolic drug design

Retrometabolic drug design takes into account the anatomical, transporter and enzymatic features of the eye whilst designing the prodrug.⁷¹⁻⁷³ Instead of studying the metabolism of the prodrugs after development, this approach predicts the metabolic route based on the desired structure and the target environment, and designs the prodrug accordingly. Formation of toxic metabolites can be avoided as the metabolic pathway leading to non-toxic metabolites is imposed on it by design. Metabolism-based drug design consists of two major concepts.^{71, 73-75}

4.1. Chemical delivery systems

Chemical delivery systems (CDS), or more appropriately, 'chemical enzyme targeting systems', involve structurally modifying the drug moiety, taking into consideration the environment to which it is to be targeted, which includes the biological membranes, the enzymes and the transporters. The drug moiety is attached covalently to a targeting function

(T) responsible for site specificity and the 'lock-in', and a modifier function (F) preventing premature metabolic conversions and protecting the active functions. As the CDS reaches the site of action, it will undergo step-wise sequential metabolic conversions releasing F and T (Figure 4).^{71, 73, 75}

4.1.1. Oxime and methoxime analogs of β -adrenergic blockers—Beta-adrenergic blockers are one class of topical anti-glaucoma agents associated with cardiovascular and respiratory side effects induced by systemic exposure through conjunctival and nasal absorption.^{76, 77} Site-specific delivery of these beta-blockers can be achieved by using the retrometabolic approach to designing enzyme-activated chemical delivery systems. The oxime and methoxime prodrugs are prepared from their β -alcohols by using the Pfitzner-Moffat oxidation.^{71, 78-84} They are sequentially converted to their corresponding alcohols in the enzyme-rich iris-ciliary by the carbonyl reductases that are responsible for the reduction of aldehydes and ketones into their corresponding alcohols.⁷⁸ The intermediate ketones as well as the active β -blockers were detected in the various eye compartments at different time intervals following topical administration.⁷⁸ It was observed, however, that even the oximes (R, = H) were not sufficiently stable in aqueous solution to provide the required shelf life in the formulation. Methoximes were synthesized and studied as an enzymatically active and stable counterpart of the oxime derivatives. Alprenoxime has a t_{90} of 2-3 months, whereas its methoxime analog showed a significantly improved t_{90} of over 1 year.⁷¹ Timolone oxime and timolone methoxime showed an increased reduction in intra-ocular pressure (IOP) in comparison to timolol maleate when applied topically in normotensive rabbits.⁸⁰ IOP-lowering effects of the oxime derivatives of both betaxolol and propranolol were greater than the corresponding beta-blocker. Topical administration of Propranolone oxime 1% showed a more pronounced IOP lowering effect than Propranolol 1%, approximately 6 h compared to 4 h, without any irritant action on the eye.⁸¹ A drawback with the topical administration of β -blockers is systemic absorption/effect through the nasal, oropharyngeal and gastrointestinal mucosa.⁸⁵ However, the oxime and the methoxime analogs of β -blockers did not show cardiovascular effects even when administered intravenously to rabbits and rats.⁸⁰⁻⁸²

4.2. Soft drugs

Prodrugs are inactive precursors of the active drug moiety that may be released by a series of sequential metabolic reactions, whereas soft drugs (SD) are active drug molecules that undergo metabolism in a predictable and controlled manner to generate an inactive moiety.^{71, 73, 74, 86, 87} The SD is an analog of the target lead drug, but its metabolic behavior is different. The SD strategy involves identification of major metabolic pathways of the active moiety, followed by the design of the molecule. Once the major metabolic pathways have been identified, one of the inactive metabolites is selected for further drug design. Novel structures are designed from this metabolite such that the soft drug so formed is isosteric and/or isoelectronic with the target lead drug and this novel structure (SD) will lead to the formation of the predetermined metabolites *in-vivo* (Figure 4).^{71, 73, 86, 87}

A number of glucocorticoids have been used in the treatment of diseases such as psoriasis, allergies, asthma, rheumatoid arthritis and lupus.^{88, 89} Many of these glucocorticoids,

however, cannot be used in the eyes because of unwanted side effects such as cataract formation and elevated IOP.⁹⁰⁻⁹² Soft glucocorticoids rapidly form predictable inactive metabolites, helping in the reduction of the unwanted side effects because the duration and extent of the exposure to the glucocorticoids is reduced.⁹³⁻⁹⁸

Loteprednol etabonate (LE), a metabolically labile ester used in the treatment of ocular inflammation and swelling, was designed by Bodor et al.⁹⁵ LE is quickly hydrolyzed to 1-cortienic acid etabonate and then into 1-cortienic acid in biological systems, thereby minimizing the likelihood of side effects.^{95, 99} Druzgala et al.⁹⁶ found the highest ratio of the metabolites to unchanged drug in the cornea, followed by the iris-ciliary bodies and aqueous humor, suggesting that the cornea was probably the main site of LE metabolism. Once systemically absorbed, LE is converted to its inactive 17-carboxylic acid metabolite.

The phenylacetic acid metabolite of metoprolol was used to design the soft drug adaprolol.⁷⁴ Adaprolol produced a prolonged and significant reduction in IOP and did not show any effect in the contralateral eye when applied unilaterally, indicating systemic inactivation.¹⁰⁰ A study comparing the IOP-reducing effect of adaprolol and timolol was conducted on 67 patients with IOP greater than 21 mmHg. This trial demonstrated that adaprolol showed a safer systemic profile while reducing IOP by approximately 20%, whereas timolol reduced IOP by 25-30%.⁷⁴ In a study by Dunham et al.¹⁰¹, timolol was observed to reduce the contralateral eye IOP by 3.1 mm Hg in 12 volunteers after a topical dose, and a reduction in 3.9 mm Hg IOP after a lingual dose (placing the dose on the subject's tongue) over a period of two h, which suggested that contralateral eye reduction in IOP was caused by systemic absorption of timolol.

5. Lipid prodrugs

Lipid molecules are a vital component of the cellular make-up of living cells. Conjugation of a drug molecule with a lipid graft would change the pharmacokinetic/pharmacodynamic profile. Manipulation of factors such as the lipid chain length, the configuration of the double bonds, and the nature of the lipid-drug linkage would aid in the design of a variety of lipid prodrug systems.^{102, 103}

5.1. Lipid conjugated -transporter targeted prodrug approach

Gokulgandhi et al.¹⁰⁴ worked on a transporter-targeted lipid-prodrug approach. Cidofovir is used in the treatment of cytomegalovirus (CMV) retinitis. The molecule, however, has low oral bioavailability. Previously, researchers had synthesized lipid-conjugates of cyclic Cidofovir (CDV) to improve its oral bioavailability.^{105, 106} Cheng et al.¹⁰⁷ studied the intravitreal pharmacokinetics of crystalline octadecyloxyethyl-cyclic-Cidofovir (ODE-cCDV) in rabbit eyes. An intravitreal injection produced 2 to 3 weeks of constant drug levels in the retina-choroid. The difference is that the crystalline drug ODE-cCDV forms a depot in the vitreous, slowly releasing the drug into the vitreous humor for a prolonged period of time. This free ODE-cCDV is then metabolized in the retinal membrane by the phosphatases releasing CDV.¹⁰⁷ The prodrug depot in the vitreous humor resulted in toxicity and the formation of retinal floaters which could affect patient compliance.¹⁰⁸ Ma et al.¹⁰⁹ used lipid-derivatized CDV, hexadecyloxypropyl-cidofovir (HDP-CDV), to form micelles in

water without the addition of any other therapeutic components. HDP-CDV demonstrated much superior protection and treatment efficacy compared to CDV in an equimolar dose in an HSV-1 retinitis model in rabbits. HDP-CDV showed a vitreous half-life of approximately 6 days, which would provide approximately 40 days of vitreous residence if 6 half-lives would clear 95% of the drug from the vitreous humor. A long vitreous half-life can act as a promising marker for prolonged therapeutic effect, as demonstrated by a 9-week prophylaxis success.¹⁰⁹

Ganciclovir is an acyclic 2'-deoxyguanosine analogue used by immunocompromised patients in the treatment of hCMV.¹¹⁰ Janoria et al.¹¹¹ studied the effect of a sodium-dependent multiple vitamin transporter (SMVT) on biotin-conjugated ganciclovir uptake in a human retinal pigmented epithelium cell line as well as in rabbit retina. They observed that SMVT recognized the biotinylated ganciclovir and also that the prodrug had a better pharmacokinetic profile in comparison to ganciclovir.¹¹¹ Cholkar et al.¹¹⁰ synthesized long chain acyl ester lipid-conjugated prodrugs of ganciclovir based on the hypothesis that conjugation of long carbon chain esters would provide sustained ganciclovir levels due to the slow hydrolysis of its ester linkages. The *in-vitro* toxicity of Ganciclovir-conjugated long chain lipids was studied in the human retinal pigment epithelial cell line (ARPE-19), showing that the prodrugs were non-toxic and well-tolerated.¹¹⁰ Gokulgandhi et al.¹⁰⁴ hypothesized a synergistic strategy to improve the permeability of cyclic CDV by attaching a lipid raft to the biotinylated drug. The lipid raft would enhance the prodrug-membrane interaction whereas the vitamin conjugation would improve SMVT transporter targeting. The hypothesis was that the enhanced lipophilicity of the prodrug system could improve melanin binding in the retina and aid in the formation of a drug depot for prolonged activity. An *in-vitro* uptake study was conducted to trace the affinity of the transporter (SMVT) for the biotinylated lipid prodrug. The results indicated that prodrugs showed strong interaction with the SMVT transporter and that the elongation of the lipid chain increased interaction with the cell membrane (B-C12-cCDV > B-C6-cCDV > B-C2-cCDV). The authors suggest that cellular uptake of both the biotinylated and non-biotinylated lipid prodrug was similar, where the lipid raft enhanced prodrug-membrane protein interaction resulted in the docking of biotin into the SMVT transporter.¹⁰⁴

As with ganciclovir, both the lipid prodrug approach and the transporter-targeted approach have been studied for acyclovir. Vadlapudi et al.¹¹² worked on the biotinylated lipid-drug conjugation strategy for acyclovir (Figure 5) and synthesized biotin-ricinoleic acid-acyclovir (BRACV) and biotin-12-hydroxystearic acid-acyclovir (B-12HS-ACV). Biotinylated lipid prodrugs showed an enhanced *in-vitro* cellular uptake in Human Corneal Epithelial Cells (HCEC) in comparison to just the biotinylated prodrugs of acyclovir, which enhanced lipophilicity and SMVT transporter targeting.¹¹² Lipid-rafting also improved the stability of these prodrugs in the cornea, iris ciliary bodies and lens, probably due to slower enzymatic hydrolysis.¹¹³

5.2. Cytarabine crystalline lipid prodrug

Cytarabine is a chemotherapeutic agent that inhibits cellular proliferation by inhibiting DNA synthesis. It demonstrates better *in-vitro* anti-proliferative efficacy in retinal pigment

epithelium than 5-Fluorouracil (5-FU).¹¹⁴ Cytarabine has an intravitreal half-life of approximately 12 h, whereas cytarabine encapsulated in a liposomal formulation has a half-life of a few days.¹¹⁵ Kim et al.¹¹⁶ synthesized two prodrugs of cytarabine, HDP-P-Ara-C (Hexadecyloxypropyl cytarabine 5'-monophosphate) and HDP-cP-Ara-C (hexadecyloxypropyl cytarabine 3',5'-cyclic monophosphate), which, following an intravitreal injection, release small amounts of drug over a prolonged period of time. *In-vitro* simulation studies revealed that the non-cyclic prodrug formed micelles and was rapidly cleared (by day 6), whereas the cyclic analog HDP-cP-Ara-C did not form micelles and showed peak release at around day 10, with the compound still detectable at the end of the experiment (day 36). *In-vitro* cytotoxicity studies determined that both compounds possess more potent anti-proliferative activity than unmodified Cytarabine.¹¹⁶

6. Co-drug systems

The co-drug strategy can be described as two temporarily-linked drug moieties with completely different pharmacophores acting synergistically to produce the same or different pharmacological actions. Prodrug systems have an inactive group attached to the active drug moiety, but co-drugs or mutual drugs have two active moieties covalently linked to each other. The drugs could be linked by a spacer molecule that could impart chemical stability or controlled drug release. The co-drug system should be non-toxic, chemically stable, non-immunogenic and non-antigenic; allow optimum loading; and provide the desired therapeutic effect without accumulating in the body.^{117, 118}

Cynkowska et al.¹¹⁹ synthesized co-drugs of ethacrynic acid (ECA) with the β -adrenergic blocking agent atenolol (ATL). Both ECA and β -adrenergic blocking agents can be co-administered as a single delivery system and could cause an overall reduction in IOP by different mechanisms. ECA and ATL were linked with ester bond links, hydrolysable at physiological pH. The ECA-ATL co-drug had a half-life of approximately 30 min in human serum and 14 h in buffer at pH 7.4.¹¹⁹

Berger et al.¹²⁰ explored a slow delivering, surgically implantable, pellet system of 5-fluorouracil (5-FU) and dexamethasone (DX) conjugate for one week and an injectable intravitreal sustained release suspension of 5-FU and TA conjugate for one month for the management of experimental proliferative vitreoretinopathy (PVR) in a rabbit model. The co-drug system was conjugated via a carbonate bond. The DX-5-FU conjugated pellet released DX and 5-FU over one week *in-vitro* and reduced the incidence of moderate retinal detachment from 70% to 20% by day 13; however, an increase in the retinal detachment rate to 40% was observed by day 20. The TA-5-FU conjugate suspension was relatively less soluble and gave a comparatively longer *in-vitro* release than the DX-5-FU pellet system, reduced the incidence of moderate to severe retinal detachment from 89% to 30% by day 14 and maintained the retinal detachment rate below 30% until day 28, showing that the prolonged release of 5-FU and triamcinolone may be more effective than the release of dexamethasone and 5-FU over a shorter period of time.¹²⁰

Mitomycin C (MMC) is used for post-operative treatment of the pterygium and also as an adjunctive treatment in glaucoma therapy. Corticosteroids such as Triamcinolone Acetonide

(TA) possess anti-proliferative properties and prevent inflammatory mediators from reaching a disease site, and can be co-administered with other drugs.^{121, 122} A MMC-TA conjugate, prepared using glutaric acid, was studied by Macky et al.¹²³ The conjugate had a half-life of approximately 23 h in aqueous solution. The anti-proliferative activities of MMC-TA and MMC were concentration-dependent, with IC₅₀ values of 2.4 and 1.7 μ M, respectively. Thus, MMC-TA appears to be a slow releasing delivery system that can be used to treat various proliferative diseases. Histopathology and electroretinogram data collected at 5 and 20 days post-intravitreal administration of the conjugate did not reveal any evidence of toxicity.¹²³ Cardillo et al.¹²⁴ synthesized pellets of covalently-linked naproxen and 5-FU and tested for the treatment of proliferative vitreoretinopathy in a rabbit model for trauma associated with tractional retinal detachment. The pellets were shown to release naproxen as well as 5-FU over a period of 30 days in an *in-vitro* release study, thus showing a sustained drug release profile, and inhibited the progression of PVR in the rabbit model. Electroretinography and light microscopy data did not suggest any toxic effects in a traumatic PVR rabbit model following treatment with a 5-FU Naproxen implant in the vitreous base.¹²⁴

7. Transporter targeted systems

The expression of a variety of nutrient transporters has been identified in ocular tissues. Researchers have harnessed the natural mechanism of nutrient transport to enhance permeation across biological membranes.¹²⁵ The drug molecules are either chemically modified or bound to a ligand or a moiety that serves as the substrate for the transporters. Transporters present on the ocular surface include influx transporters such as amino acid transporters (LAT1 and LAT2), glucose transporter (Glut1), monocarboxylate transporter (MCT), and peptide transporters (PEPT1 and PEPT2).^{24, 125-127} The most widely studied influx transporters for the delivery of ophthalmic drugs include peptide transporters, monocarboxylate transporters, organic cation transporters, organic anion transporters, the neutral (0) and cationic (+) amino acid transport systems, the organic anion transporting polypeptide family and the sodium- dependent multivitamin transporter. Efflux proteins are also present on the ocular membranes and expel substrates from inside the cells, or from the cell membranes, thus reducing the ocular bioavailability of substrates.¹²⁵ The efflux transporters belong to the ATP-binding cassette (ABC) family of transporters and are mainly represented by P-glycoprotein (P-gp), multidrug resistant proteins (MRP) and breast cancer resistant proteins (BCRP).^{24, 125, 127-130}

7.1. Peptide transporters

These proton-dependent membrane transporters mainly translocate certain peptides (mono, oligo, dipeptides, tripeptides) and peptidomimetics across membranes.^{126, 131} They have been classified as PepT1 and PepT2 (peptide transporters) and peptide/histidine transporters (PHT1 and PHT2).^{132, 133} Drugs/drug candidates having different chemical structures and pharmacological activities may be delivered intracellularly by these transporters.^{134, 135} Several molecules have been tested that target peptide transporters for translocation through ocular membranes.^{131, 134-140} Majumdar et al.¹⁴¹ studied the vitreal pharmacokinetics of various dipeptide monoester ganciclovir prodrugs (L-tyrosine-L-valine, L-glycine- L-valine

and L-valine-L-valine) and the interaction with the peptide transporter in the rabbit retinal tissues.¹⁴¹ The study demonstrated improved permeability of the dipeptide prodrugs into the retinal pigment epithelium in comparison to the mono-peptide prodrug, valine-ganciclovir.¹⁴¹

7.2. MCT

The monocarboxylic acid transporters MCT-1 and MCT-3 are present on the conjunctival, corneal and retinal epithelia and on the ciliary, choroid and retinal pigment epithelium.¹²⁷

Vooturi SK et al.¹⁴² formulated and synthesized various derivatives of gatifloxacin (GFX), such as gatifloxacin dimethyl amino-propyl, (DMAP-GFX), carboxy-propyl (CP-GFX) and amino-propyl (2-methyl) (APM-GFX), targeting OCT, MCT, and ATB (0, +) transporters, respectively. The prodrugs were more lipophilic than their respective parent compounds.¹⁴² DMAP-GFX exhibited 12.8-fold greater solubility than GFX and was selected for further *in-vivo* studies. Compared to GFX, DMAP-GFX showed 1.4-, 1.8-, and 1.9-fold improvement in permeability across the cornea, conjunctiva, and SCRPE, respectively.¹⁴² DMAP-GFX and CPGFX prodrugs showed higher transport across rabbit cornea and SCRPE and bovine conjunctiva. The *in-vivo* studies revealed 3.6- and 1.95-fold higher levels of DMAP-GFX in the vitreous humor and choroid retinal pigmented epithelium (CRPE), respectively, compared to GFX, 1 h post-topical administration. This study successfully used the prodrug derivatization strategy to enhance solubility and OCT-mediated delivery of gatifloxacin to the back of the eye.¹⁴²

7.3. Overriding efflux transporters by prodrug derivatization

The efflux of drug molecules may be overcome by targeting the influx transporters through prodrug derivatization, thus improving ocular bioavailability because of improved epithelial penetration. Therapeutic agents covalently bonded to a transporter-targeted ligand may translocate through the influx transporters and circumvent the efflux pumps, resulting in the desired action at lower doses.^{143, 144}

Sheng Y et al.¹⁴⁵ studied the effect of amino acid and dipeptide prodrugs with respect to their ability to bypass P-glycoprotein (P-gp)-mediated cellular efflux of prednisolone. Cellular uptake studies carried out in the MDCK-MDR1 cell line indicated a higher efflux of prednisolone by P-gp compared to its prodrugs. Erythromycin was chosen as the model substrate and [¹⁴C]-Erythromycin uptake was studied in the presence and absence of prednisolone and its prodrugs. The results suggested that the dipeptide prodrug valine-valine prednisolone (VVP) did not significantly alter erythromycin uptake, indicating a lower affinity of VVP towards P-gp.¹⁴⁵ Interaction of prednisolone and its dipeptide prodrug with the peptide transporters was studied through [³H]-GlySar uptake studies in MDCK-MDR1 cells. The results suggested that 100 μ M VVP diminished the uptake of [³H]-GlySar to 40%, whereas no significant inhibition was observed in the presence of the same concentration of prednisolone.^{145, 146} The authors extrapolated these data to conclude that peptide transporters could generate higher transcorneal absorption. The efflux ratio (for transepithelial permeation studies across MDCK-MDR1 cells) of prednisolone was 2.2, indicating significantly lower apical to basolateral transport (A to B) in comparison to the basolateral to apical transport (B to A), signifying that the transport of prednisolone was

limited by P-gp. In contrast, the A to B transport of VVP was 2.6-fold higher than the A to B transport of prednisolone, proving both circumvention of P-gp as well as the influx of prodrugs mediated by peptide transporters.^{145, 147}

Katragadda S et al.¹⁴³ investigated the modulation of efflux mechanisms using transporter-targeted prodrug derivatization of a model P-gp substrate, quinidine. L-valine and L-valine-valine ester prodrugs of quinidine were synthesized.^{143, 148} The results demonstrated that prodrug derivatization of quinidine can modulate P-gp-mediated efflux. These prodrugs exhibited a reduced or diminished affinity towards P-gp compared to the parent molecule quinidine and were recognized by the peptide transporter. The authors report increased uptake of the mono- and dipeptide prodrugs compared to quinidine. Transport studies were carried out using isolated rabbit corneas at 34°C for 3 h in the presence and absence of an inhibitor (glycylsarcosine). Both prodrugs, valine-quinidine and valine-valine-quinidine, were inhibited, indicating their interaction with the oligopeptide transporters. The enhanced permeability of the prodrug suggested that this could be a viable strategy to overcome P-gp-mediated efflux.¹⁴³

P-gp-, BRCP- and MRP-mediated cellular efflux has been reported to be involved in resistance mechanisms.¹⁴⁹ P-gp substrates can be co-administered with P-gp inhibitors, but the high inhibitor doses required might result in toxicity. Patel et al.¹⁵⁰ have previously demonstrated the potential of large neutral amino acid transporter targeting in human prostate cancer cells. The L-isoleucine ester prodrug of quinidine (Ile-quinidine) showed a higher intracellular accumulation in comparison to quinidine.¹⁵⁰ Subsequently, the authors examined the effect of modification of a P-gp substrate with a large neutral amino acid transporter-targeted promoiety, and examined whether this conjugation circumvented the P-gp efflux pump. These studies were carried out with and without Cyclosporine A and GF 120918, as a model P-gp substrate/inhibitor and P-gp inhibitor, respectively, in MDCK-MDR1 cells with [14C]-erythromycin as a model substrate. Enhanced uptake of [14C]-erythromycin took place in the presence of cyclosporine A and GF 120918 and also quinidine (25 and 50 µM), whereas Ile-quinidine (25 µM) did not have any effect. Moreover, the authors observed that the apical to basolateral transport of quinidine increased dramatically in the presence of cyclosporine A and GF 120918, whereas Ile-quinidine did not have any such effect. The results suggest that unlike quinidine, Ile-quinidine is not a P-gp substrate.¹⁵¹

Prednisolone, a topically-applied glucocorticoid that exerts its anti-inflammatory effect by binding to glucocorticoid receptors, is also a P-gp substrate with limited ocular bioavailability. Amino acid and peptide prodrugs of Prednisolone have been synthesized by Sheng et al. to improve its aqueous solubility and also to aid in circumventing P-gp mediated efflux. It was observed that prodrugs such as valine-valine-prednisolone showed affinity towards peptide transporters and provided a feasible strategy to overcome P-gp mediated efflux.¹⁴⁵

8. Hydrophilic prodrug strategies

The prodrug approach can be utilized to improve the aqueous solubility of the therapeutic candidates. Ocular tissue targeted molecules need to be soluble in the tear fluid as well as be able to permeate across the alternating lipophilic and hydrophilic layers of the corneal ultrastructure, thus demanding a delicate balance between hydrophilicity, lipophilicity and solubility. While an increase in the degree of branching or the length of the acyl side chain results in lipophilic prodrugs, incorporation of an ionizable group increases aqueous solubility. Ester-based promoieties such as dicarboxylic acid hemi-esters (e.g., hemi-succinates), phosphate esters, sulfate esters and α -amino esters, can be utilized to enhance the hydrophilic characteristics of the molecule.^{31, 152}

8.1. Hemi-esters / dicarboxylic acid esters

Cannabinoids have very poor aqueous solubility and thus efforts need to be focused on developing a stable formulation which can be instilled topically.¹⁵³ Several derivatives of Tetrahydrocannabinols (THC) have been tested for their IOP-lowering effect in different vehicles such as mineral oil and sesame oil, wherein mineral oil-based formulations showed a superior IOP-lowering effect. Many of the tested derivatives, apart from Delta-9-THC and 11-OH-Delta-9-THC, were found to be inactive in reducing the IOP.^{154, 155} However, *in-vitro* corneal permeability of THC from light mineral oil was only 1.86×10^{-8} cm/s.¹⁵⁶ Because of THC's high lipophilicity and poor water solubility, a prodrug approach could be utilized to improve corneal penetration of this compound.

Hingorani et al.¹⁵⁷ tried to improve the aqueous solubility of THC by synthesizing dicarboxylic acid prodrugs of THC, THC-hemisuccinate (THC-HS) and THC-hemiglutamate (THC-HG), and also observed the effect of ion pairing on the *in-vitro* transcorneal permeability of these prodrugs.¹⁵⁷ The prodrugs demonstrated pH-dependent solubility and exhibited a 6-fold increase in transcorneal permeability in comparison to THC at pH 5. This is probably because the major fraction of the prodrug exists in the unionized state at pH 5. Ion-pairing with 1-arginine and tromethamine shielded the negative charge at physiological pH, and the ion-paired prodrugs showed a 7-fold increase in transcorneal permeability. THC-HG was rapidly converted to THC with a half-life of approximately 71, 25, 47.5, 144.7, and 36.7 minutes in the cornea, aqueous humor, iris-ciliary bodies, vitreous humor and retina-choroid, respectively.¹⁵⁷ The authors also compared the *in-vivo* ocular disposition of THC from mineral oil-based formulations versus surfactant and ion pair-based formulations containing THC-HG.¹⁵⁷ THC in mineral oil did not deliver any detectable levels of THC into the aqueous humor and iris-ciliary bodies. In contrast, the THC-HG surfactant formulation delivered 32.1 ± 12.6 ng/100 μ L to the aqueous humor and 35.6 ± 12.5 ng/50 mg to the iris ciliary bodies, and the THC-HG ion pair formulation delivered 52.2 ± 18.7 ng/100 μ L to the aqueous humor and 93.1 ± 41.4 ng/50 mg to the iris-ciliary bodies. Thus, the chemically modified THC, THC-HG, had improved physicochemical, solubility and solution stability properties, resulting in increased penetration into the aqueous humor and deeper ocular tissues following topical administration.¹⁵⁸

8.2. Phosphate prodrugs

The phosphate prodrug strategy can also be employed to increase the hydrophilicity of poorly water-soluble compounds. The water solubility of the phosphate prodrugs is imparted by the di-anionic phosphate moiety, the cleavage of which can be catalyzed by alkaline phosphatases to release the lipophilic parent molecule.³¹

Retinoblastoma is an intra-ocular malignancy observed in children, initiated by the loss of the Retinoblastoma 1 (RB1) gene, affecting the developing retina.¹⁵⁹ Spleen Tyrosine Kinase (SYK) is upregulated in retinoblastoma and is required for tumor cell survival.¹⁵⁹ Targeting SYK with small molecule inhibitors resulted in caspase-mediated cell death, both *in-vitro* and *in-vivo*.¹⁵⁹ R406, N4-(2,2-dimethyl-3-oxo-4-pyrid[1,4]oxazin-6-yl)-5-fluoro-N2-(3,4,5-trimethoxyphenyl)-2,4-pyrimidinediamine, is an inhibitor of spleen tyrosine kinase (SYK). However, R406 has low aqueous solubility, which encouraged the design of a methylene-phosphate prodrug of R406, fostamatinib (R788). Fostamatinib is an SYK inhibitor in phase II clinical trials as an oral therapeutic agent for the treatment of inflammatory disorders such as rheumatoid arthritis.¹⁶⁰⁻¹⁶² Pritchard et al.¹⁶³ administered R406 subconjunctivally in pre-clinical mouse models of retinoblastoma. Subconjunctival R406 did not show efficacy, due to insufficient intraocular exposure. At similar total doses, the more water soluble prodrug R788 in a cosolvent solution of 5% Cremaphor-EL, 0.5% ethanol, 0.2% Tween 80 and 94.3% PBS achieved a maximum vitreous concentration 6-fold higher than the water insoluble R406 free base in emulsion, suggesting that aqueous solubility controls maximal equilibrium ocular drug concentrations.¹⁶³

Retinoblastoma tumors are highly vascularized and can be treated by targeting blood vessels.¹⁶⁴ Combretastatin A-4 phosphate (CA-4P) is a water-soluble phosphate prodrug that can be used to target tumor vasculature.^{165, 166} CA-4P displayed dose-dependent inhibition of neovascularization in a murine oxygen-induced proliferative retinopathy model with no apparent side-effects.¹⁶⁷ Escalona-Benz et al.¹⁶⁴ studied the effect of sub-conjunctival CA-4P on tumor vasculature and growth in a murine model of hereditary retinoblastoma and observed that CA-4P induced a dose-dependent reduction in vessel density and significant tumor reduction in treated eyes compared to placebo-treated control eyes.¹⁶⁴

9. Conclusion

Extensive research is being carried out with the goal of improving drug delivery to the ocular tissues. The use of different strategies, including formulation as well as prodrug derivatization, has shown great results in enhancing ocular bioavailability of the lead candidates/drugs and in overcoming absorption barriers. Several novel strategies, such as chemical, drug-polymer and retro-metabolic targeting and designing, discussed in this review, target new prodrug technologies in an effort to improve the fate of the drug in ocular tissues. Along with the transporter-targeted approach, soft drugs, pro-prodrugs, lipid prodrugs and co-drugs are some of the new techniques that have shown promise with respect to delivering drugs to not only the anterior but also to the posterior segments of the eye. Table 1 provides a summary of all the prodrug derivatization strategies covered in the review.

10. Expert opinion

Prodrug derivatization is a versatile and feasible approach for imparting desired biopharmaceutical characteristics to potential drug candidates. This approach can be tailor-made to improve solubility, stability or permeability characteristics of the lead molecules, without causing any damage to the biological barriers involved. Prodrugs have also been designed to target sites of action and also to control release rates of the parent moiety.

Despite the vast amount of research being conducted in the field of prodrug derivatization, only a few marketed products exist, highlighting the complex discovery and development steps involved. At the very outset, the prodrug design concept will be limited to only those lead candidates that allow derivatization, through the addition of promoieties, that impart the desired characteristics. Secondly, the prodrug has to have sufficient stability in the ophthalmic formulation, solutions and suspensions being the most preferred, in order to achieve desired finished product shelf-life. Ester and amide bonds, commonly used in prodrug design, have short half-lives in an aqueous environment, such as in aqueous solutions and suspensions, due to chemical hydrolysis. Formulation approaches, such as use of surfactants and cyclodextrins, to shield the labile linkages, or use of cosolvents, may increase the stability of some prodrugs. The sensitivity of the ocular tissues, however, may limit the stabilizing approaches. Additionally, commonly used strategies for other routes of administration, such as lyophilized powders for reconstitution, may not be feasible, in terms of use and cost, for ophthalmic formulations because of the low volume that is used per application for local use (topical, intravitreal or peri-ocular applications). It should be noted, however, that recent developments in ophthalmic formulation strategies provide a few options wherein the exposure of the prodrug to the aqueous environment can be reduced through the use of emulsions and colloidal nanoparticulate systems, such as lipid nanoparticles.

In addition to the above challenges, variability in enzymatic bioreversion of the prodrug could also be a cause of concern at the clinical stage. Such a variability could arise because of genetic polymorphism or external factors such as competition/interactions with other substrates that lead to variations in enzyme activity. Some other factors that could affect ocular tissue enzyme levels include age, race and disease states. Changes in enzyme levels could lead to variations in the bioreversion rates which could, in turn, lead to differences in duration and intensity of therapeutic activity. This is especially significant for prodrugs as the therapeutic activity is dependent on the release of the parent active molecule through enzymatic and chemical processes. Also, inter-species variability in enzyme expression and activity may confound prediction of activity in humans.

In spite of all the above challenges, prodrug derivatization can be successfully used with some lead candidates as evident from a number of commercially available products. Some of the prodrug design strategies discussed in this article, such as soft drugs, co-drugs and pro-prodrugs (Table 1) may be applicable to only few cases and will require a detailed optimization process to ensure efficacy and minimize variability. Other approaches, such as derivatization to modulate the hydrophilicity of the parent drug and transporter targeting, are comparatively straight-forward and easy to achieve. It should be noted that the success of the

transporter targeting approach will depend on the capacity and specificity of the targeted transporter. A judicious combination of prodrug design and formulation development approaches could, however, provide some solutions to the challenges faced in ocular drug delivery.

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Fig. 1.

Biological barriers of the eye (tight barriers are indicated in red, others in green; route of elimination). The main pathway for drugs to enter the anterior chamber is via the cornea (1). Some large and hydrophilic drugs prefer the conjunctival and scleral route, and then diffuse into the ciliary body (2). After systemic administration small compounds can diffuse from the iris blood vessels into the anterior chamber (3). From the anterior chamber the drugs are removed either by aqueous humor outflow (4) or by venous blood flow after diffusing across the iris surface (5). After systemic administration drugs must pass across the retinal pigment epithelium or the retinal capillary endothelium to reach the retina and vitreous humor (6). Alternatively, drugs can be administered by intravitreal injection (7). Drugs are eliminated from the vitreous via the blood–retinal barrier (8) or via diffusion into the anterior chamber (9).

Figure 2.

Supramolecular nanofibers of Triamcinolone Acetonide

1. The first step involves a one-step synthesis of Succinated Triamcinolone Acetonide from parent drug Triamcinolone Acetonide, in the presence of pyridine at room temperature for 3 h.
2. The second step involves formation of TA hydrogel from Succinated TA in PBS buffer solution (pH = 7.4) for about 35h at 37°C.

Figure 3.

Conversion of Pro-prodrug/ double prodrug UNIL088 to Cyclosporine A by formation of pSer-Sar-CsA as intermediate by enzymatic and chemical reactions.

Figure 4.

The retrometabolic design loop

1. Chemical delivery systems (CDS) structurally modify the active drug moiety by attaching covalently to a target function (T) responsible for site specificity and a modifier function (F), to prevent premature metabolic conversions. On approaching the site of action, CDS undergo metabolic conversions releasing F, and T.
2. Soft Drug strategy involves selection of one of the inactive natural metabolites of the drug for retrometabolic design resulting in formation of the soft drug, which after metabolism gives the same inactive metabolite.

Figure 5.

Biotinylated Lipid prodrugs: Biotin-12-Hydroxystearic acid-ACV. The drug moiety, Acyclovir, is conjugated to the biotinylated lipid raft, 12- Hydroxystearic acid. The lipid raft enhanced prodrug-membrane protein interaction and the biotin conjugation improved the SMVT transporter targeting.

Table 1.

A compilation of experimental and marketed prodrugs employed in the treatment of various ophthalmic diseases.

Ophthalmic diseases	Prodrugs used	References
Glaucoma: IOP lowering effect	Prostaglandins: Latanoprost, bimatoprost, travoprost,	129,
	Cannabinoids: –9 THC prodrugs	157, 158
	Adrenergic agonists: Epinephrine prodrugs, e.g. Dipivefrine	40
	Beta-blockers: Oximes of Alprenolol, Betaxolol	82-84
Herpes Simplex Keratitis (HSV)	Peptide and amino acid prodrugs of Acyclovir, Ganciclovir	135-138
Corneal graft rejection, dry eye disease	Cyclosporine prodrugs, e.g. UNIL088	62-65, 68
Proliferative vitreoretinopathy	N-alkoxycarbonyl prodrugs of 5-fluorouracil, Hexadecyloxypropyl cytarabine 5'-monophosphate, Hexadecyloxypropyl cytarabine 3',5'-cyclic monophosphate	120, 116
Ocular pain and inflammation	Non-steroidal anti-inflammatory agents like Nepafenac	41, 42
Retinoblastoma	Combretastatin A-4 Phosphate	164-167
Age-related Macular degeneration (AMD)	4-Chloro-3-(5-methyl-3-[[4-(2-pyrrolidin-1-ylethoxy)phenyl]amino]-1,2,4-benzotriazin-7-yl)phenyl Benzoate (TG100801)	168

Table.2

Summary of prodrug strategies described in the review.

Strategies used	Description	Sub-types	Ref
Polymeric delivery systems	Polymeric prodrug systems involve the active drug moiety being covalently bound to the polymeric backbone which would also be attached to a solubilizing molecule and a targeting function specific to the biological targets. Thus, the active agent would be released only when the functionalized polymer reaches the desired site of action.	PEG-based prodrugs	46-48
		Molecular hydrogels	52-56
		Lactic acid based polymers	57,58
Pro-prodrug/ Double prodrug	The double-prodrug, or the pro-prodrug strategy, involves derivatizing the prodrug for site specific enzymatic hydrolysis, which would then lead to spontaneous release of the parent drug at the target site and an inactive prodrug at other sites.		60,62-65,68
Retrometabolic drug design	Retrometabolic drug design takes into consideration the routes of drug deactivation, or activation, while designing the prodrug. In a chemical delivery system, the drug moiety is attached covalently to a targetor function (T) responsible for site specificity and the “lock-in”, and a modifier function (F), preventing premature metabolic conversions and protecting the active functions. As the chemical delivery system reaches the site of action, it undergoes step-wise sequential metabolic conversions releasing F, and T. A soft drug, on the other hand, is a novel structure, designed from an inactive metabolite, such that the soft drug so formed is isosteric and/or isoelectronic with the target lead drug and this novel structure (soft drug) will lead to the formation of the predetermined metabolite in-vivo.	Chemical delivery systems	80-83
		Soft drugs	95-98
Lipid-based delivery systems	Lipid-prodrugs involve conjugation of a drug molecule with a lipid draft whilst playing with variables such as the lipid chain length, configuration of double bonds, and the nature of the lipid-drug linkage.		104-113
Co-drug systems	Co-drug strategy can be described as two temporarily linked drug moieties with completely different pharmacophores acting synergistically to produce same or different pharmacological action.		119,120,123,124
Transporter targeted delivery	The drug candidates /drug can be targeted to nutrient transporters in order to enhance permeation across biological membranes. These drug candidates are either chemically modified, or bound to a ligand or a promoiety, such that prodrug serves as a substrate for the nutrient transporters. Efflux transporters are also present on the ocular membranes and expel substrates from inside the cells, or from the cell membranes, thus reducing the ocular bioavailability of drug molecules that are substrates of these efflux proteins. Drugs delivered by covalent bonding to a transporter targeted ligand may translocate through the influx transporters and circumvent the efflux pumps, resulting in the desired action at lower doses.	Peptide transporter targeted prodrugs	132,134-141
		Monocarboxylate transporter - targeted prodrugs	125-127,142
		Over-riding Efflux transporters	145-148
Hydrophilic prodrug strategies	The low-aqueous solubility issue of a water insoluble parent drug can be overcome by converting it into a soluble prodrug by conjugation with hemi-succinates, phosphate group, amino-acids or other water soluble promoieties.	Dicarboxylic acid prodrugs	156-158
		Phosphate ester prodrugs	163-167