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Given that cyclosporine (CsA) is poorly water soluble and currently marketed products are not well tolerated, novel approaches for safe and efficient CsA delivery to the eye are of great interest.

Modern approaches to the ocular delivery of cyclosporine A

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Cyclosporine A (CsA) has long been the mainstay treatment for dry eye syndrome (DES), one of the most common disorders of the eye. However, the poor water solubility of CsA renders it difficult to formulate it into topical ocular dosage forms. Restasis® is currently the only US Food and Drug Administration (FDA)-approved CsA formulation, while Ikervis® has recently been launched in Europe, with both commonly associated with severe ocular discomfort. Therefore, several CsA formulations have been investigated with the aim to improve bioavailability while reducing adverse effects associated with the marketed formulations. In this review, we summarize recent advances in ocular CsA delivery that provide safer and more effective alternatives for the management of DES and other ocular inflammatory conditions.

Introduction

CsA is a metabolite of the fungi *Tolypocladium inflatum* and *Beauveria nevus* that was initially suggested for use as an antifungal agent. Its immunosuppressive activity soon became evident and, because of the reduced incidence of associated myelotoxicty, CsA eventually became the mainstay treatment after organ transplantation [1,2]. Systemic CsA is still used, although to a lesser extent, to treat several other autoimmune diseases, including those with eye involvement [3]. CsA is the preferred immunomodulatory agent for topical treatment of several immune-mediated ocular surface disorders [4], and its prevalence in the treatment of these ocular disorders is second only to corticosteroids, whose adverse effects are well documented [5]. CsA therapy has been approved for the treatment of keratoconjunctivitis sicca (KCS), more commonly known as dry eye syndrome (DES). It is also frequently used off-label to treat several other ophthalmic conditions, such as posterior blepharitis [6,7], ocular rosacea [8,9], vernal keratoconjunctivitis [10–14], atopic keratoconjunctivitis [15–17], acute corneal graft rejection [18], and conjunctival graft versus host disease [19–21].

DES is one of the most prevalent ocular surface disorders, and is usually characterized by increased evaporation or decreased production of tear fluid, resulting in damage to the inter-palpebral ocular surface and moderate to severe discomfort [22]. Symptoms of DES have been reported in approximately one out of seven individuals above the age of 48, with its prevalence nearly doubling after 59 years of age [23–25]. A recent study estimated that nearly 20% of

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hospitalized patients above the age of 50 had DES, with old age and illiteracy being major predictors of the disorder [26]. On the recommendation of the International Dry Eye Workshop in 2007, DES was classified as a multifactorial disease of the tears and ocular surface that can be triggered by a variety of underlying causes. However, recently, there has been increasing evidence that inflammation has a key role in the manifestation of DES. Ocular surface abnormalities, such as the appearance of inflammatory cell intermediates in the lacrimal gland and an increase in immune-related antigens and cytokines at the conjunctival epithelium, are commonly demonstrated by both autoimmune (Sjögren's syndrome) and non-autoimmune (non-Sjögren's syndrome)-mediated DES [27–30], making treatment of the underlying cytokine–receptor-mediated inflammatory processes the primary 'causative therapeutic approach' [31,32]. A combination of symptomatic therapy, which includes modification of the ocular environment (by increasing humidity, occlusion of lacrimal canaliculi, or simulation of tears), and pathogenic treatments, including the use of antibacterial and anti-inflammatory agents (corticosteroids, antihistamines, tetracyclines, and CsA), is currently recommended for DES therapy [32].

CsA is the anti-inflammatory agent of choice for the treatment of DES, because it can be used long term without adverse effects commonly associated with other anti-inflammatory agents, such as steroids [5]. Furthermore, unlike corticosteroids, the activity of CsA results from specific and reversible action on T cells, making it safe for prolonged use. For example, corneal epitheliopathy and eyelid maceration associated with long-term CsA therapy were found to be reversible with complete cessation on discontinuation of the drug [33]. The immunomodulatory activity of CsA helps in reducing the inflammation associated with subconjunctival and lacrimal glands, resulting in increased goblet cell density and tear production [34,35]. CsA tends to bind with specific nuclear proteins that initiate the activation of T cells, thus preventing T cell production of inflammatory cytokines and disrupting the immune-mediated inflammatory response [31,36]. CsA is a hydrophobic molecule and, therefore, is difficult to formulate into conventional topical ocular delivery systems. Significant research has been performed over recent years to develop safe and effective ocular delivery systems for CsA. In this review, we highlight recent efforts to improve the ophthalmic delivery of CsA in terms of its bioavailability and ocular tolerability while reducing adverse effects.

Conventional CsA formulations

Systemic CsA is generally not considered for the treatment of ocular pathologies because of severe systemic adverse effects, such as nephrotoxicity and hypertension [2,37], although significant concentrations of CsA have been reported in tears and lacrimal glands after oral administration [38]. Thus, the topical route is generally preferred because, as well as reducing systemic adverse effects, it also helps to achieve improved bioavailability and specific targeting to the ocular tissues [39,40].

Dose-ranging randomized clinical trials have shown that topically applied CsA is effective at concentrations between 0.05 and 0.1% (w/v). No additional benefits were observed at higher concentrations; hence, clinical trials are generally recommended at a maximum concentration of 0.1% [4,41]. However, because CsA is a neutrally charged and hydrophobic molecule (Fig. 1) with an aqueous solubility of less than 10 μg/ml (0.001%) at physiological temperature [42], formulation of aqueous eye drops at these concentrations is difficult. Attempts have been made to improve the aqueous solubility of CsA using surfactants and/or penetration enhancers, such as macrogolglycerol ricinoleate (Cremophor EL®) and benzalkonium chloride, with the latter also commonly used as a preservative in ocular formulations [43]. Although these excipients can improve the solubility and penetration of CsA, their use is limited by their high irritation potential because these molecules typically function by compromising the integrity of the ocular tissues [44–46].

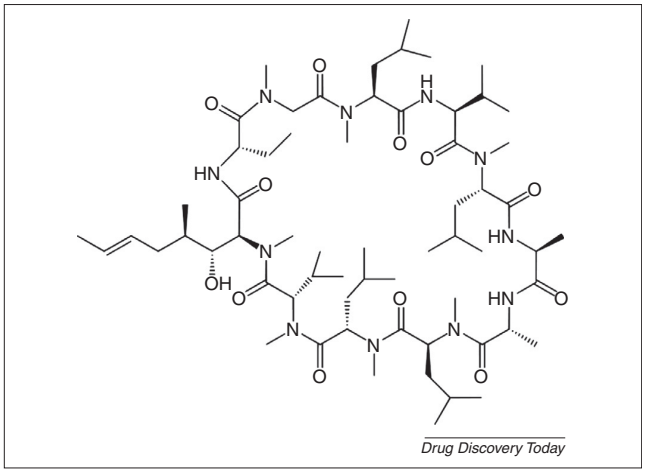

FIGURE 1

Molecular structure of cyclosporine A (CsA), a cyclic undecapeptide with very low aqueous solubility.

CsA solutions in vegetable oils were considered as the next best alternative for topical administration to the eye and concentrations as high as 2% could be achieved [47,48]. Despite their poor absorption, oily CsA solutions have demonstrated success in the treatment of DES by improving tear production and inducing regression of corneal neovascularization in a canine model [49–54]. Similar results were also observed in humans after topical application of 2% CsA in olive oil [55,56]. However, one of the major limitations of vegetable oils in ophthalmic preparations is the increased incidence of ocular toxicity after frequent use, with blurring of vision also commonly observed because of the high viscosity of the carrier oils. BenEzra *et al*. [38,57,58] showed that penetration of CsA from olive oil drops was negligible in normal or slightly inflamed eyes, but increased significantly over time because the corneal barrier was compromised as a result of toxic effects of the oily vehicle. Thus, the use of topical CsA oily drops for the management of ophthalmic conditions has largely been discontinued.

CsA ointments [59–61] and oil-in-water (o/w) emulsions [62] have also been used to reduce the toxicity associated with oily solutions, although they have not been able to eliminate it completely. Currently, Restasis® (Allergan) is the only CsA formulation approved by the FDA for DES therapy in humans. It is a 0.05% CsA emulsion of castor oil in water and is often associated with severe adverse effects, such as ocular burning (most common), conjunctival hyperemia, discharge, epiphora, eye pain,

foreign body sensation, pruritus, stinging, and visual disturbance [63]. Devecı *et al*. [64] recently showed that ocular irritation associated with Restasis usually decreases within 1 week of continuous therapy and significant resolution of DES can be observed at the 1-month follow-up. A veterinary ointment of 0.2% CsA (Optimmune®, Schering-Plough Animal Health) has also been approved for DES therapy in dogs and, although it has been shown to resolve the symptoms of DES, associated ocular toxicity, probably because of the oily base, is significant [65,66]. Ointments are generally more viscous and have a cloudy appearance and, thus, tend to blur the vision, which, in addition to their tolerability issues, reduces patient acceptability. Recently, a cationic nanoemulsion containing 0.1% CsA was launched in Europe for the treatment of severe DES under the brand name Ikervis® (Santen). Unlike emulsions and ointments, this system does not cloud vision because of its low viscosity; however, adverse effects, such as stinging and pain, have frequently been reported [67].

The limitations of currently approved formulations leave tremendous scope for the development of improved ocular formulations for the safe and efficient delivery of CsA to the eye. The focus of new CsA technologies has predominantly been the improvement of solubility, transcorneal penetration, and precorneal residence time, with a simultaneous reduction in the frequency of dosing and irritation potential. Such CsA formulations could significantly improve patient comfort and compliance, thus increasing the quality of life of patients with DES.

Recent approaches in CsA delivery

Over the past few years, several novel strategies have been suggested for delivering CsA to the ocular tissues, with topical, episcleral, subconjunctival, and intravitreal routes being investigated. Of these, topical administration remains the most preferred route for the treatment of DES, because it is non-invasive, painless, and convenient. For the purpose of this review, the key approaches to improving ocular drug delivery of CsA have been classified into three major areas of research: (i) chemical modification of the drug; (ii) novel application of excipients; and (iii) novel ophthalmic dosage forms.

Chemical modification of the drug

Reversible chemical modification of CsA to obtain prodrugs with improved aqueous solubility was first suggested by Hammel *et al*. [68] when they coupled CsA with diketopiperazine to synthesize dipeptide esters with improved oral bioavailability. Monomethoxy(polyethyleneglycol) derivatives of CsA have also been suggested for improving solubility and absorption of CsA in oral preparations. However, esterification of the free hydroxyl group of CsA with a solubilizing moiety is the generally accepted approach for prodrug synthesis for ocular applications. Lallemand *et al*. [69,70] developed a series of amphiphilic acidic prodrug molecules (patented by DeBiopharm, Switzerland) having an approximately 25 000 times higher solubility than CsA in isotonic phosphate buffer solution (PBS) at pH 7. These prodrugs are quantitatively hydrolyzed in artificial tears to release CsA within 1 min. Prodrug conversion into the parent molecule was significantly faster in tear fluid than in a buffer at physiological pH, indicating that the hydrolysis is enzyme mediated. Aqueous formulations of these esterified CsA prodrugs were well tolerated and have shown

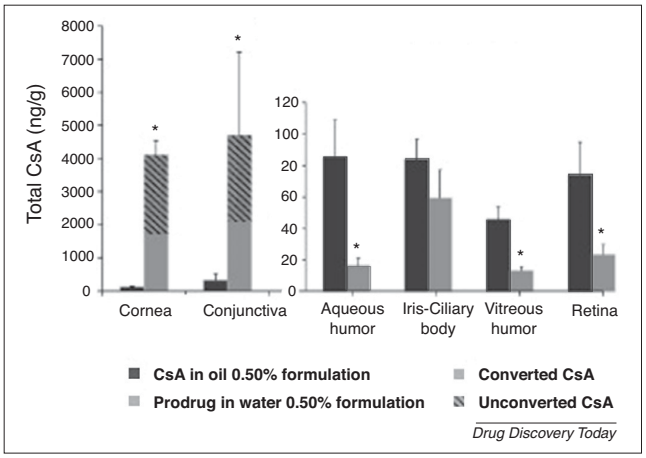

FIGURE 2

Ocular distribution of cyclosporine A (CsA) in oil and CsA prodrug in water. Prodrug formulations significantly increased the amount of CsA absorbed by, and accumulated in, the cornea and conjunctiva; however, no improvement was observed in the penetration of CsA across the cornea.
Source: Adapted from [75].

a significant improvement in basal tear production in DES [71,72]. Aqueous prodrug solutions have also been evaluated for their efficacy in the treatment of corneal graft rejection and it was found that prodrug eye drops applied five times a day were therapeutically equivalent to a 10 mg/kg/day intramuscular injection in rats [73]. Recent studies have shown that 2% prodrug solutions have a 200–500-fold higher conjunctival permeability than the conventional 2% CsA in oil formulation. Accumulation of CsA prodrug formulations in the cornea to form large tissue deposits that provide a sustained release effect over prolonged periods of time was also observed. However, prodrug formulations did not show much improvement in the permeability across the cornea and into the aqueous humor compared with the conventional CsA emulsion, probably because of the rapid conversion of the prodrug into CsA at the corneal surface (Fig. 2). This depot formation could have the added advantage of overall poor systemic absorption of prodrug formulations, reducing the incidence of systemic complications and immunosuppression. Prodrug formulations with CsA concentrations higher than 2% are currently under investigation for safety and toxicity [74,75].

Novel application of excipients

Several new excipients have been introduced to improve CsA solubility in ocular formulations and enhance their bioavailability (Table 1).

Cyclodextrins

The introduction of cyclodextrins (CDs) in ophthalmic drug delivery is relatively recent; however, they have rapidly gained popularity because of their pronounced benefits in drug solubilization and stabilization. CDs are cyclic oligosaccharides of six (α-CD), seven (β-CD), or eight (γ-CD) glucopyranose units with a hydrophobic cavity in the center. In aqueous solutions, water from the hydrophobic cavity is displaced by hydrophobic molecules, resulting in the formation of large water-soluble complexes. CDs have been used on several occasions to prepare aqueous CsA

TABLE 1

Novel excipients used in ocular CsA delivery systems.

Excipients	Dosage form	Advantages	Limitations	Refs
Cyclodextrins	Aqueous eye drops	Solubilize CsA in aqueous formulations; show improved bioavailability and low toxicity at optimized concentrations	Cannot be used in concentrated solutions; ocular toxicity can be observed at higher concentrations because of complexation of cellular components	[76–79]
Semifluorinated alkanes	Eye drops (CyclASol®)	Safe and well tolerated; solubilize hydrophobic drugs to form clear eye drops; improved penetration; no preservative required; lubricating nature	No sustained release effect	[80,81]
Cationic vectors (amines, chitosan, poly-L-lysine, Eudragit)	Emulsions, liposomes, nanoparticles	Improve precorneal residence time because of mucoadhesion	Amine vectors can cause stability problems; safety and toxicity is a concern because ocular irritation is usually observed with most cationic moieties	[95]

eye drops with significant improvement in transcorneal penetration and retention [76–79]. α-CDs have been found to be the most efficient in solubilizing CsA, because the cavity size is a good fit for the cyclic CsA molecule. When used to prepare concentrations below 0.025%, α-CDs improved CsA bioavailability without demonstrating any toxic effects *in vivo* [77,79].

Semi-fluorinated alkanes (SFAs)

SFAs are a class of amphiphilic fluorinated compounds that are physically, chemically, and biologically inert and capable of dissolving several hydrophobic drugs. Although they have been used intraocularly as intravitreal tamponades for several years, their use in drug delivery to the front of the eye is relatively recent [80]. Given their established safety profile, excellent spreading properties, and ability to solubilize and stabilize several hydrophobic drugs, SFAs can be used as suitable carriers for ocular preparations. The added advantages of SFAs include their ability to form clear solutions that do not cause any blurring of vision or ocular discomfort while also providing lubrication to the ocular surface, which is particularly useful in DES treatment. Moreover, because these solutions are nonaqueous, they do not require any preservatives, surfactants, or pH modifiers, which are frequently implicated in ocular toxicity. Recent studies showed that SFA-based solutions containing 0.05% CsA were well tolerated and significantly increased CsA penetration across the cornea and into the aqueous humor when compared with Restasis, rendering SFA-based formulations promising candidates for the treatment of ocular inflammatory conditions [81]. This technology is currently registered as CyclASol® (Novaliq GmbH, Germany) and recently conducted Phase I clinical trials have shown promising results [82], with the company now recruiting patients for a Phase II clinical trial [83].

Positively charged vectors

One of the major objectives of topical CsA delivery has been to improve ocular bioavailability of the drug by increasing corneal penetration and precorneal residence time. Therefore, positively charged ocular formulations have received much interest because of their higher mucoadhesion and, thus, longer precorneal residence time. Studies performed by Daull *et al.* [84] showed that cationic emulsions have significantly greater bioavailability than Restasis, which is an anionic o/w emulsion. Safety profiles of cationic and anionic emulsions were further compared and, while their tolerability was similar, only the cationic emulsion was able to maintain the normal healing rate of the human corneal epithelium *in vitro* and reduce inflammation *in vivo* [85]. Similar to most epithelia, corneal and conjunctival cells are negatively charged at physiological pH and, therefore, cations can adhere to and penetrate them more easily [86,87]. Their cell membranes are further coated with a layer of mucin containing negatively charged sialic acid groups, which develop electrostatic interactions with positively charged vectors and thus improve the precorneal residence time [88].

Stearylamine has been used extensively to impart a positive charge to liposomes [89–91] and ocular emulsions [92–94] to increase mucoadhesion and, thus, retention. Oleylamine is another cationic lipid that has been used for preparation of cationic ophthalmic emulsions [88]. However, a major disadvantage of these amines is their poor stability and compatibility with other excipients, while ocular tolerability of amines remains a significant concern. Hence, chitosan has emerged as the cationic agent of choice in ocular formulations. Chitosan is a biodegradable and biocompatible linear polysaccharide with several positively charged free amino groups that interact with mucin. There is some evidence that, in addition to the positive charge, the specific nature of chitosan might also be responsible for improving the uptake of drug molecules. Calvo *et al.* [95] showed that the bioavailability of nanocapsules coated with chitosan was at least twofold higher than for poly-L-lysine-coated nanocapsules. However, commercialization of chitosan formulations might be difficult, because raw materials of natural origin often demonstrate high batch-to-batch variability. Recently, several studies also reported that, despite its biodegradability, chitosan can display some toxicity, especially when administered as nanoparticles [96,97], while chitosan hydrogels of a certain molecular weight have also shown to initiate an inflammatory response and, thus, delay wound closure in a corneal scrape wound model [98]. Therefore, Eudragit®, a synthetic polymer frequently used for the preparation of enteric oral dosage forms, has been suggested as an alternative for ophthalmic formulations [99]. Eudragit is a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate groups. Eudragit-based ocular colloidal formulations have shown good tolerability and

TABLE 2

Novel ophthalmic dosage forms for CsA delivery to the front of the eye.

Drug delivery system	Advantages	Limitations	Refs
Micelles	Improved uptake into all layers of the cornea; sustained release over extended periods	Generally poor shelf life; irritation potential and toxicity can be significant	[103–106]
Liposomes			
Liposomes	Form depots in cornea for sustained drug release over prolonged periods	Short half-life at the corneal surface results in poor transcorneal penetration	[113,114,118]
Proliposomes	Improved stability and simple manufacturing procedure	Safety and irritation potential need to be assessed	[119]
Nanoparticles			
Nanospheres/nanocapsules	Improved retention and uptake into cornea; reduced drug toxicity can be observed	Difficult to scale up; can show some long-term toxicity	[133,134]
Nanoemulsions	Improved uptake because of transport by transcellular pathway	Generally poor stability and manufacturability	[142]
Solid lipid nanoparticles	Improved bioavailability; improved shelf-life compared with nanoemulsions	Generally poor long-term stability and manufacturability	[143–145]
Other			
In situ gelling systems	Increased precorneal residence time and sustained release	High burst release; blurring of vision can reduce patient compliance	[154,157,160]
Hydrogels	Sustained release of the drug over prolonged periods	Discomfort and foreign body sensation; adverse effects because of poor oxygen permeability	[164,165,171]

low incidence of ocular irritation [100–102], while decomposition of Eudragit is typically slow, providing controlled drug release with a low burst effect.

Novel ophthalmic dosage forms

The field of ocular therapeutics has seen the development of several novel delivery systems in the form of micelles, liposomes, nanoparticles, *in situ* gelling systems, and hydrogels, which have also been evaluated for the delivery of CsA (Table 2 and Fig. 3).

Micelles

Micelles are self-assembling spherical colloidal systems that are frequently used for the solubilization of hydrophobic molecules. Typically, micelles are formed around a hydrophobic drug in aqueous solution because of the orientation of surfactant molecules to form a hydrophobic core enclosing the drug within a hydrophilic shell. *N*-Octyl chitosan has been used as a surfactant for preparation of CsA micelles for DES therapy [103]. CsA uptake from these micelles was found to be significantly higher than from

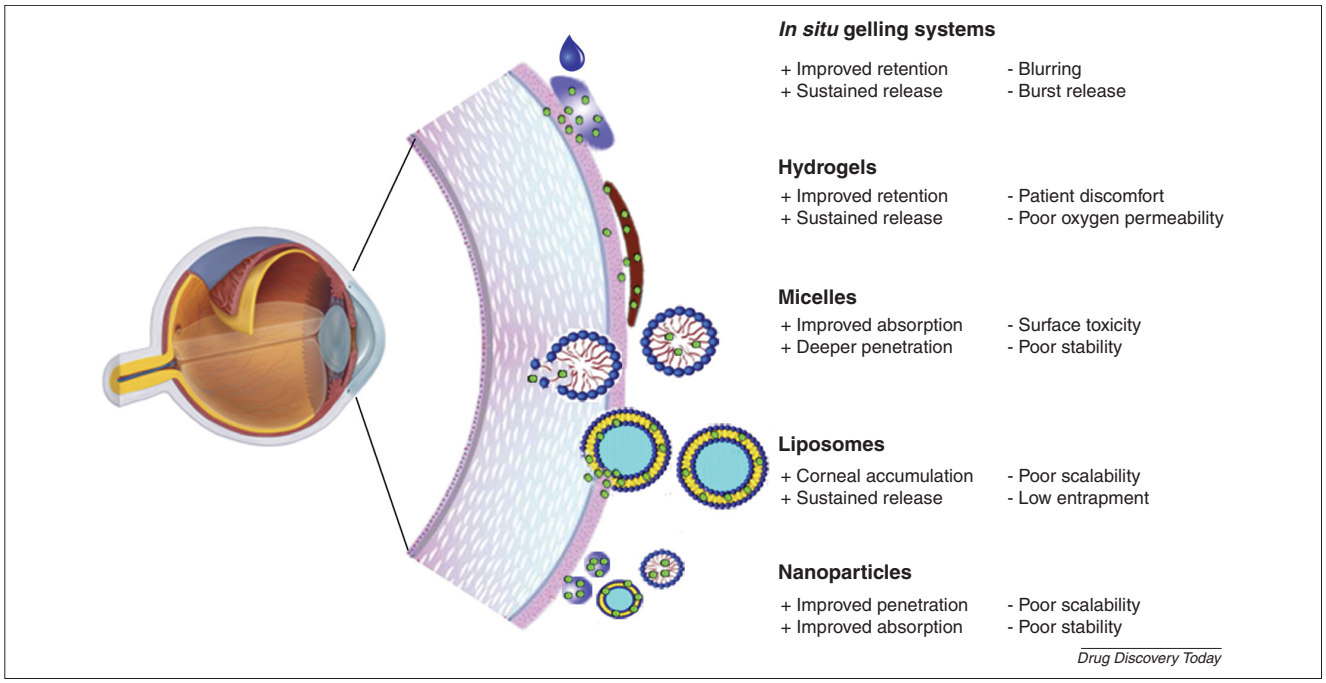

FIGURE 3

Schematic representation of novel ophthalmic dosage forms used to improve the bioavailability of topically applied cyclosporine A (CsA).

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