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Review article

Cyclosporine A delivery to the eye: A pharmaceutical challenge

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Abstract

Systemic administration of cyclosporine A (CsA) is commonly used in the treatment of local ophthalmic conditions involving cytokines, such as corneal graft rejection, autoimmune uveitis and dry eye syndrome. Local administration is expected to avoid the various side effects associated with systemic delivery. However, the currently available systems using oils to deliver CsA topically are poorly tolerated and provide a low bioavailability. These difficulties may be overcome through formulations aimed at improving CsA water solubility (e.g. cyclodextrins), or those designed to facilitate tissue drug penetration using penetration enhancers. The use of colloidal carriers (micelles, emulsions, liposomes and nanoparticles) as well as the approach using hydrosoluble prodrugs of CsA have shown promising results. Solid devices such as shields and particles of collagen have been investigated to enhance retention time on the eye surface. Some of these topical formulations have shown efficacy in the treatment of extraocular diseases but were inefficient at reaching intraocular targets. Microspheres, implants and liposomes have been developed to be directly administered subconjunctivally or intravitreally in order to enhance CsA concentration in the vitreous. Although progress has been made, there is still room for improvement in CsA ocular application, as none of these formulations is ideal.

Keywords: Cyclosporine A; Ocular delivery; Intraocular; Topical; Delivery system; Prodrug; Review

1. Introduction

Cyclosporine A (CsA) is a cyclic undecapeptide produced by *Tolypocladium inflattum Gams* and other *fungi imperfecti*. This drug is now routinely used as an oral immunosuppressor for organ transplantation. It acts by selective inhibition of interleukin-2 release during the activation of T-cells and causes suppression of the cell-mediated immune response [1]. The resultant immunosuppression is non-toxic and reversible when treatment is stopped. Therefore, most of the diseases that involve cytokines or immune–related disorders are potential targets of CsA.

Over the past years, CsA has been evaluated for numerous potential applications in ophthalmology. It is effective in the treatment of severe intraocular inflammations affecting the posterior segment of the eye, when administered systemically by i.v. injection [2] or orally [3]. Systemic CsA has also shown efficacy in peripheral ulcerative keratitis associated with Wegener's granulomatosis [4], in severe Grave's ophthalmopathy [5] and it was also effective in preventing recurrence of graft rejection after keratoplasty, and this for a long period of time [6]. In fact, intraocular fluids (aqueous or vitreous humor) and extraocular organs or annexes (cornea, conjunctiva and lachrymal glands) can be reached through the systemic pathway after oral or i.v. administration. CsA concentrations of 25–75 μg/ml were measured in human tears after an oral daily administration of 5 mg/kg [7] but deleterious side effects such as nephrotoxicity and hypertension may occur [8–10]. Although it has met numerous difficulties, topical ocular delivery should offer a good alternative. Despite a poor intraocular penetration, topical CsA has been successfully used in a variety of immune-mediated ocular surface phenomena like vernal conjunctivitis [11], dry eye syndrome [12] and the prevention of corneal allograft rejection [13]. An ideal topical ocular formulation must fulfill several requirements: the formulation must be well

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tolerated and easy to administer, increase CsA residence time in the eye and avoid systemic absorption (the toxic concentration in blood is above 300 ng/ml [14]). In addition, the formulation should have a long shelf life and be manufactured easily. The main difficulty is that CsA cannot be prepared in formulations based on the commonly used aqueous ophthalmic vehicles because of both its hydrophobicity (log $P = 3.0$ [15]) and its extremely low aqueous solubility (6.6 μg/ml [16]). Therefore, in most studies, CsA was dissolved and administered in vegetable oils [17–19]. However, these media are poorly tolerated, result in relatively low ocular availability, and have short shelf lives. The concentration that has been mostly investigated for an eyedrop solution is 1% w/v [20,21] but concentrations ranging from 2% w/v [22] to 0.05% w/v [23] have also been explored. In all cases, the concentration of the formulation remains secondary as long as therapeutic levels in the ocular tissues are achieved by the dosage form, immune response and inflammation being suppressed at a concentration of 50–300 ng/g of tissue [24]. When high intraocular concentrations are needed, the drug is also injected directly into the eye or periocularly (by the subconjunctival route). Numerous formulations were developed to avoid repeated injections and achieve controlled release of CsA or to enhance efficacy of topical administration. This review summarizes the main pharmaceutical systems and devices that have been described for topical and intraocular delivery of CsA to the eye. It will first present and discuss the topical systems developed during the last 15 years. In the second part, intraocular and subconjunctival devices will be reviewed.

2. Topical administration

Most ocular medications may be administered topically in order to treat surface as well as intraocular disorders. This route is often preferred for the management of various pathological diseases that affect the anterior chamber of the eye, for two main reasons: it is more conveniently administered and provides a higher ratio of ocular to systemic drug levels. To be administered topically and to achieve the necessary patient compliance, CsA must present a good local tolerance. Topical CsA in olive oil solution induces a burning sensation and an irritative effect on the conjunctiva. These side effects have been attributed to the vehicles used [25]. Patients did not complain of such disorders after application of a 2% w/w CsA ointment, and ocular examination revealed no significant lesions [26]. A recent study [27] showed that formulations of CsA in peanut oil were non-toxic to rabbit eyes. However, an unusual corneal deposit [28] was reported in a patient after 5 days of topical use of a 1% w/v CsA olive oil eyedrop. This deposit was probably due to the precipitation of CsA on the corneal surface. No long-term study of surface toxicity is yet available.

New developments in the topical delivery of CsA can be divided in two general areas of research: new delivery systems (solutions, ointments, colloidal carriers and drug-impregnated contact lenses) and chemical modifications of the drug (prodrugs).

2.1. Solutions and ointments

2.1.1. Oil solutions and ointments

Several vegetable oils such as arachis [29], castor [30], olive [7] and peanut oils [31] have been used to solubilize CsA. Petrolatum oils (Cremophor [32]) and ointments ([23,33] have also been investigated as CsA vehicles for topical eye administration. Some authors [24,34] have reported that such formulations could achieve, after topical administration, therapeutic levels in ocular tissues: Kaswan [24] reported concentrations of 4 μg/g in the cornea and 60 ng/g in the iris 2 h after application. On the other hand, a majority of authors [7,17,30,35] have reported none or negligible intraocular penetration. Furthermore, oils are known to be poorly tolerated by the eye and are therefore rapidly evacuated from the ocular surface. Due to its lipophilicity [15], CsA has a greater affinity for the vehicle than for the cornea, providing a low local availability. Also, these vegetable oils may present problems of stability such as rancidity [36]. Despite these drawbacks, olive oil is still tested in the prevention of corneal graft rejection [13] and is still the most frequent reference vehicle cited. A marketed ointment formulation for veterinary use (Optimmune®, 0.2% Cyclosporine USP Ophthalmic Ointment, Schering Plough, Welwyn, Herts, UK) is available for the treatment of keratoconjunctivitis sicca and ocular surface inflammatory diseases in dogs [37]. This formulation has not reached the human field mainly because of its poor acceptability by patients. Tolerance in the veterinary area is evaluated by tests (Draize, slit lamp) that do not take into account blurred vision and patient discomfort; very important criteria in the human field.

2.1.2. Aqueous solutions

Attempts have been made to improve the solubility of CsA in water by complexation of CsA with cyclodextrins or penetrations enhancers.

2.1.2.1. Cyclodextrins. Cyclodextrins are complex sugars of cyclo-malto-hexose type, exhibiting a lipophilic center hidden by an external hydrophilic layer [38]. These physicochemical characteristics enable cyclodextrins to combine with lipophilic molecules and increase their water solubility. CsA combined to α-cyclodextrin was solubilized up to 750 μg/ml in water [39], which is approximately 100-fold higher than for CsA alone. Four different formulations were tested and the optimal concentration for maximum corneal permeability and lowest toxicity was found to be 0.025% w/v CsA in 40 mg/ml α-cyclodextrin solution. After the application of one drop

every 2 h four times a day on the rabbit eye, this solution achieved concentrations of 4.1 ± 0.4 μg/g in the cornea, which was five to 10 times higher than those obtained with a 10% w/w CsA ointment, and above therapeutic levels. This study was confirmed by Cheeks [33], who showed on excised rabbit corneas that CsA bound to cyclodextrins resulted in higher corneal penetration than an application of corn oil solutions. This formulation resulted, however, in a very small reservoir effect in the cornea, because of the low intrinsic quantity of drug in the formulation and the short residence time on the eye surface.

2.1.2.2. Penetration enhancers. Penetration enhancers are chemicals that can help solubilize CsA and transiently modify the corneal epithelium to promote drug penetration through the cornea. Azone® (laurocapram) [40] was used as a CsA solvent in order to solubilize the drug and improve its delivery to the eye. Clinically significant concentrations of CsA were measured in the grafted corneas of rabbits but little or no drug was found either in the aqueous humor or in the blood of the treated animals. CsA in Azone® resulted in suppression of the severity and incidence of graft rejection. This penetration enhancer has, since, been shown to induce cytotoxicity on corneal epithelium [41].

The effect of three other penetration enhancers on the transcorneal permeation of CsA was evaluated [42]. Flux rates of radiolabeled-CsA across human excised corneas were measured in the presence and absence of aqueous solutions of benzalkonium chloride (0.01%), dimethylsulfoxide (DMSO) (20%) or Cremophor (10% and 20%) (concentrations expressed in w/v). Cremophor and benzalkonium significantly increased flux rates of CsA across cornea (Fig. 1) while no change was observed with DMSO. Benzalkonium presented a very good tolerance at the concentration used in eye drops as preservative (0.01% w/v) [43], but induced ocular irritation at higher concentration (1% w/v) [44]. The topical application of Cremophor has been associated with changes of corneal surface structure [45] while severe anaphylactic hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy were observed after systemic absorption [46]. The use of penetration enhancers represents a potentially interesting approach, but with the serious limitation of the low tolerance of these molecules that act by modifying the corneal properties (mostly a disruption of the epithelial cell layer of the cornea).

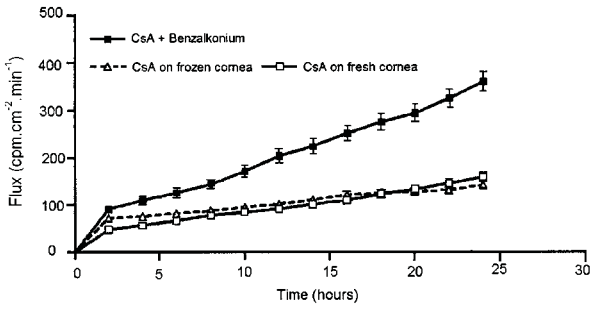

Fig. 1. Influence of benzalkonium (0.01%) on the ex vivo flux values of CsA (■) across excised fresh (△) and frozen (□) human corneas after topical application of CsA aqueous solution [43].

2.2. Colloidal carriers

Colloidal carriers are small particles of 100–400 nm in diameter, suspended in an aqueous solution. Calvo [47] has shown that colloidal particles were specifically taken up by the epithelial cells of the cornea by endocytosis. The cornea then acts as a reservoir, releasing the drug to the surrounding tissues. These carriers represent a means of delivering lipophilic drugs into hydrophilic tissues. They include micelles, emulsions, nanoparticles, nanocapsules and liposomes.

2.2.1. Micelles

CsA was solubilized at 0.1% w/v in isotonic and neutral aqueous solution by micelles of the non-ionic surfactant, polyoxyl 40 stearate, at a concentration of 2% w/v [48]. After a single administration on the rabbit eye (50 μl), this suspension provided a 60-fold higher concentration in the cornea than the 0.1% w/v CsA castor oil control solution. Although these results are promising, a certain number of points remain to be further investigated. Indeed, polyoxyethylene stearates are widely used in pharmaceutical formulations and cosmetics and are generally regarded as essentially non-toxic and non-irritant materials [49], but ocular tolerance of the surfactant is not known and has not been evaluated in this work. In addition, micelles are often unstable and their shelf life must be investigated.

2.2.2. Emulsions

Oil-in-water emulsions are particularly useful in the delivery of lipophilic drugs. In vivo data from early studies confirmed that emulsions could be effective topical ophthalmic drug delivery systems [50], with a potential for sustained drug release [51]. With the recent improvements in aseptic processing, and the availability of new well-tolerated emulsifiers (polysorbate-80), emulsion technology is currently under evaluation for topical CsA delivery. Ding and colleagues have developed a castor oil-in water microemulsion [52]. This emulsion, stabilized by polysorbate 80, solubilizes up to 0.4% w/w CsA and remains stable over 9 months at room temperature. It was found to cause only mild discomfort and slight hyperemia on the rabbit eyes when applied eight times a day during 7 days. CsA penetrated into rabbit extraocular tissues (cornea, lachrymal glands, conjunctiva) at concentrations adequate for local immunosuppression while penetration into intraocular tissues was much lower and absorption into blood was minimal [53]. These encouraging results allowed the formulation to undergo clinical trials of phase II and III

in dry eye disease [23,54]. The phase II trial performed on 162 patients demonstrated good tolerance of the emulsion and significant improvement of ocular signs and symptoms of moderate-to-severe dry eye disease [23]. CsA formulations of 0.05% and 0.1% w/w were selected for evaluation in phase III trials. In this pivotal study, RESTASIS® demonstrated statistically significant and clinically relevant increases in Schirmer wetting versus vehicle at 6 months. It has received approval (December 2002) from the United States Food and Drug Administration (FDA) for RESTASIS® (cyclosporine ophthalmic emulsion, 0.05%) as the first and only therapy for patients with keratoconjunctivitis sicca whose lack of tear production is presumed to be due to ocular inflammation.

Since epithelial corneal cells exhibit negative charges on their surface, Klang [55] hypothesized that a positively charged emulsion would interact with the corneal cells and prolong residence time on the surface of cornea. As Ding's emulsion [52] was negatively charged, Abdulrazik and coworkers [56] made a positively charged emulsion loaded with CsA. Positive charges were introduced in the emulsion through the insertion of stearylamine (0.12% w/w). Consequently, the spreading coefficient of this emulsion on the cornea was four times higher than that of the negatively charged emulsion. It was therefore deduced that the positively charged submicron emulsion has better wettability properties on the cornea. After a single dose of the positively charged emulsion on the rabbit eye, CsA yielded higher maximum concentrations in the conjunctiva and the cornea, compared to the emulsion of Ding [52] (Fig. 2). Tolerance evaluation and stability tests have to be performed but so far the results are encouraging. The emulsion should soon be submitted to a phase I clinical trial.

2.2.3. Liposomes

Liposomes are membrane-like vesicles consisting of one or more concentric phospholipid bilayers alternating aqueous or lipophilic compartments, making them potential carriers for lipophilic drugs. Milani [57] applied that technology to the ocular delivery of CsA. He obtained a 40% trapping efficiency of CsA into such vesicles. The formulation was tested topically on corneal rat allografts: a liposome suspension at a CsA concentration of 0.21 mg/ml was administered five times daily on grafted corneas. After 60 days, a 77% rate of graft survival was achieved with the CsA loaded liposomes group while only 45% survival rate was observed in the olive oil CsA solution control group (Fig. 3). As serum levels were undetectable, the authors concluded that the graft was reached only by the topical route. However, the potential of liposomes as a topical CsA delivery system remains limited because of their short half-life on the corneal surface and relatively poor stability. A charge-inducing agent like stearylamine could be introduced in order to improve intraocular penetration and drug availability, as shown by Law [58]. Furthermore, large-scale manufacture of sterile liposomes is expensive and technically challenging, which make liposomes secondary candidates for CsA delivery. Subconjunctival and intraocular injections of CsA loaded liposomes have also been investigated (see Section 3).

2.2.4. Nanoparticles

Nanoparticles, primarily developed for i.v. administration, have demonstrated promising results over the last 10 years in ophthalmology. These systems are able to encapsulate and protect the drug against chemical and enzymatic degradation, improve tolerance, increase corneal uptake and intraocular half-lives. Three main studies have been undertaken to evaluate aqueous suspensions of CsA loaded nanoparticles.

Calvo and coworkers [59] have made nanocapsules composed of an oily phase (Mygliol®) surrounded by a poly-ϵ-caprolactone (PCL) coat. CsA was loaded in the oil

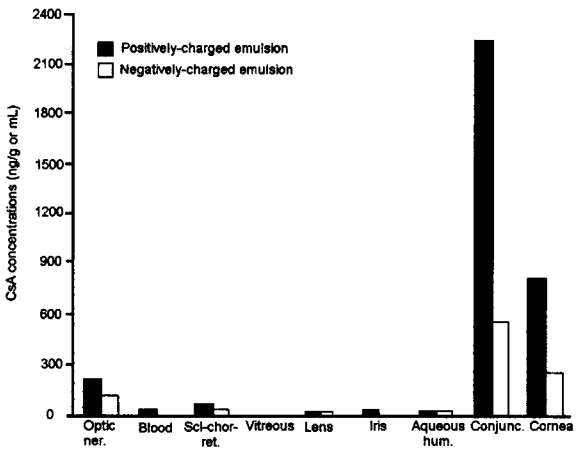

Fig. 2. Concentrations (ng/g or ml) of CsA in different ocular tissues, 60 min after topical application of the positively charged (■) and negatively charged (□) emulsions containing CsA on the rabbit eye [57].

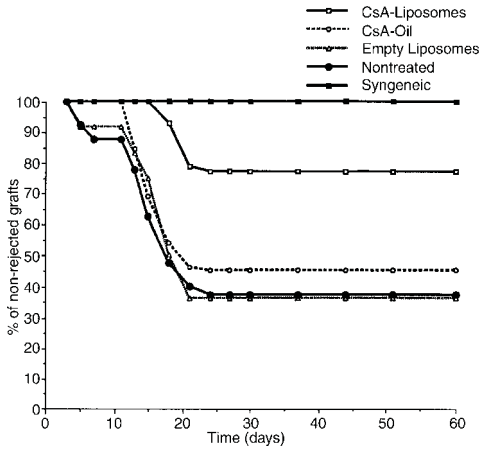

Fig. 3. Percentage of non-rejected grafted corneas after topical treatment by CsA-bounded liposomes (□), CsA in olive oil (○) and empty liposomes (△) on a rat model and comparison to controls non-treated grafts (●) and syngeneic grafts (■) over 60 days [58].

at a concentration of up to 50% (w/w CsA/PCL ratio) to give a 10 mg/ml CsA concentration in the final formulation. After topical administration, these capsules were taken up by corneal epithelial cells [60] and achieved corneal levels of CsA that were five times higher than a 10 mg/ml CsA oily solution. The delivery system was well tolerated but did not provide significant CsA levels at the ocular mucosa for an extended period of time. Consequently, this formulation failed to prolong corneal graft survival in an experimental rat model [61]. Moreover, PCL nanocapsules are degraded after autoclaving and γ-irradiation [62] and thus must be sterilized by aseptic filtration. However, since these nanocapsules have a mean size in the range of 210–270 nm the only practical alternative is a totally sterile manufacturing process. Although PCL nanoparticles did not succeed in prolonging corneal graft survival they may be useful in the treatment of extraocular diseases, as the cornea would represent a CsA reservoir.

The ex vivo corneal absorption of CsA loaded polyisobutylcyanoacrylate (PACA) nanoparticles and nanoparticles in poly(acrylic) acid (carbopol) gel was evaluated in bovine corneas [63]. The authors found that CsA concentrations in corneas were significantly higher with nanoparticles in gel than nanoparticles alone and CsA olive oil solution. However, one limitation of the ex vivo model is that it does not take into account tear wash and lachrymal drainage. Further characterization of nanoparticles (encapsulation efficiency, size and zeta potential, release rates studies) would help an understanding of the underlying physiological processes involved in transcorneal absorption. PACA nanoparticles are known to penetrate into the outer layers of the corneal epithelium causing a disruption of the cell membranes [64]. In vivo tolerance of these CsA loaded nanoparticles should be investigated.

Chitosan is a biopolymer obtained by deacetylation of chitin that is extracted from crab shells. This polymer is positively charged and biodegradable. These properties make it a good candidate for ocular delivery. Recently, an innovative colloidal drug carrier, made by ionic gelation of chitosan, has been described [65]. These nanoparticles encapsulated CsA at levels up to 9% of their weight. After four applications of 10 μl, the formulation achieved high concentrations in vivo (rabbit model) in external ocular tissues (cornea and conjunctiva) from 2 to 48 h after application (Fig. 4), while other tissues (aqueous humor, iris ciliary body, and blood) presented negligible CsA levels. These relatively large CsA concentrations in the periocular tissues are explained by the corneal and conjunctival surface retention due to the positive charges of chitosan. It should be noted that no Draize or tolerance tests have been performed to evaluate this formulation. However, Felt [66] demonstrated excellent tolerance of a topically applied chitosan gel. Since steam and dry heat sterilization affect physical properties of chitosan [67], and since sterile filtration is not possible for such nanoparticles (mean size 283 ± 24 nm), γ-sterilization should be investigated for these carriers.

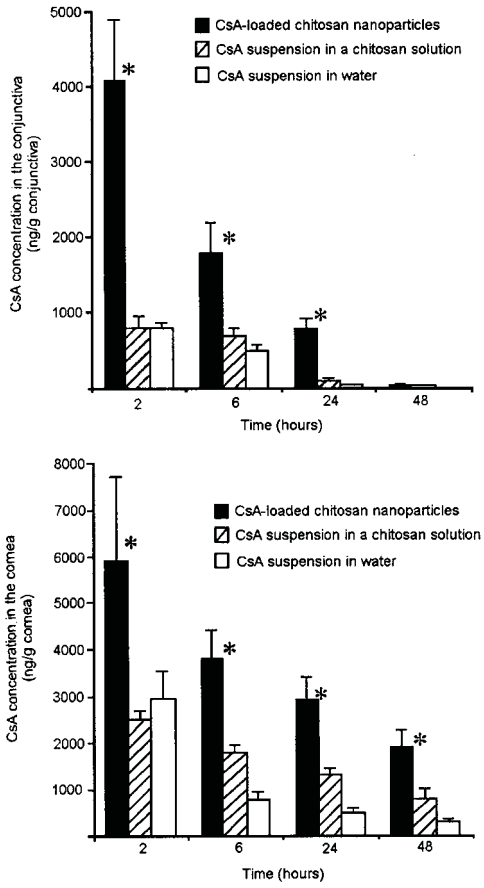

Fig. 4. CsA concentrations (ng/g) in the cornea and conjunctiva after topical application of CsA loaded chitosan nanoparticles (■) and control formulations over 48 h in a rabbit model (* statistically significant differences) [66].

Since, chitosan is a polymer of natural origin, batch to batch heterogeneity, with respect to molecular weight may complicate manufacturing.

The nanoparticle approach is not yet completely satisfactory as the precorneal clearance is still too rapid. Of the three CsA colloidal carriers described, the most promising, so far, is the chitosan carrier mainly because of the therapeutic levels achieved in periocular tissues and its good tolerance.

2.3. Solid formulations

Since most solutions are eliminated from the ocular surface within a few minutes by normal tear turnover and lachrymal drainage, solid systems have been developed in order to enhance contact time of the drug with the extraocular tissues. These include collagen-based systems.

2.3.1. Collagen shields

This approach consists of the application on the cornea of a collagen shield loaded with CsA. Reidy and coworkers

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