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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).

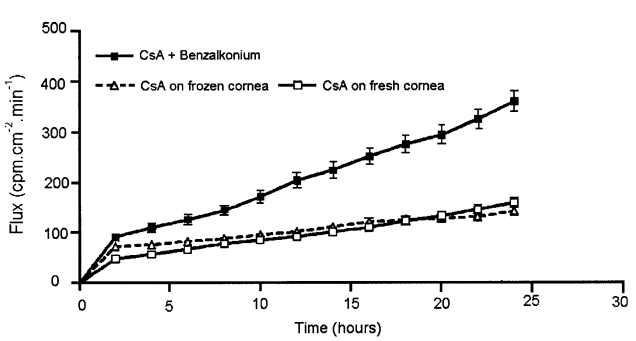

Fig. 1. Influence of benzalkonium (0.01%) on the ex vivo flux values of CsA (■) across excised fresh (△) and frozen (□) human corneas after topical application of CsA aqueous solution [43].

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

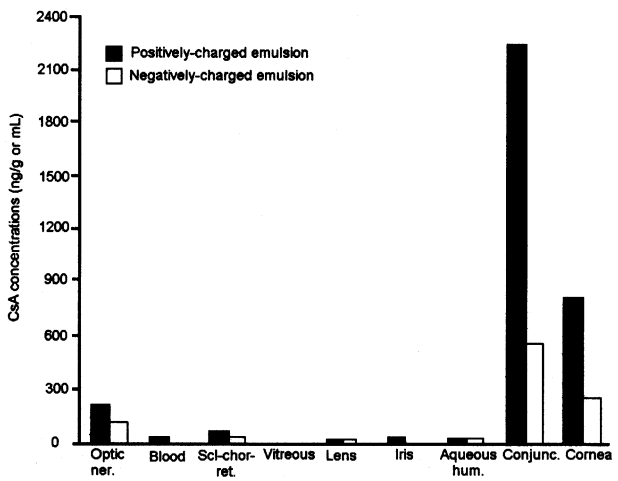

Fig. 2. Concentrations (ng/g or ml) of CsA in different ocular tissues, 60 min after topical application of the positively charged (■) and negatively charged (□) emulsions containing CsA on the rabbit eye [57].

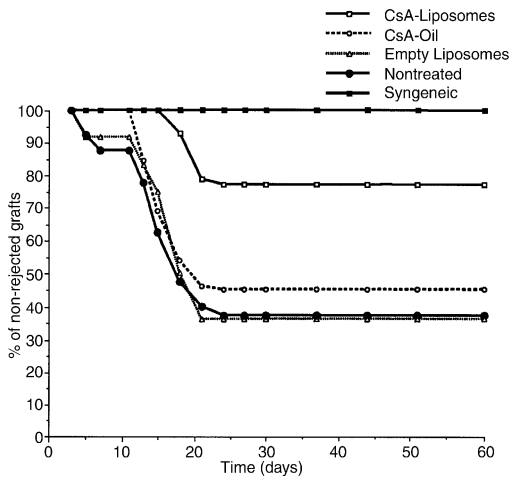

Fig. 3. Percentage of non-rejected grafted corneas after topical treatment by CsA-bounded liposomes (□), CsA in olive oil (○) and empty liposomes (△) on a rat model and comparison to controls non-treated grafts (●) and syngeneic grafts (■) over 60 days [58].

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.

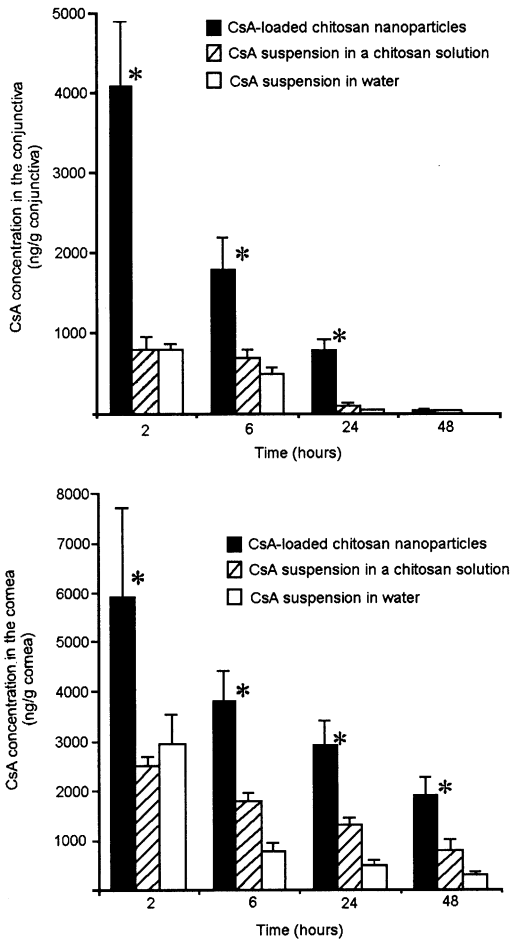

Fig. 4. CsA concentrations (ng/g) in the cornea and conjunctiva after topical application of CsA loaded chitosan nanoparticles (■) and control formulations over 48 h in a rabbit model (* statistically significant differences) [66].

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

[68] tested such a shield loaded with 4 mg CsA on rabbit cornea to enhance drug penetration into the anterior chamber by increasing contact time on the cornea. These shields are directly applied on the rabbit cornea, and the eyelids are kept closed by a piece of tape to avoid rejection of the system. The device achieved therapeutic concentrations in the cornea and aqueous humor from 2 to 8 h after application with a maximum peak at 4 h; no CsA was detected in the blood. Both the corneal and aqueous humor concentrations of CsA achieved with the shield were 10-fold higher than those obtained with topical CsA-olive oil drops and over a time period which was twice longer than the control. Collagen shields are useful as drug delivery systems and also as bandages after corneal surgery. However, several drawbacks of the system must be kept in mind. The shield may blur the vision. It is also applied dry on the eye, a maneuver that may be poorly tolerated especially in some pathologies such as dry eye. Also, the collagen matrix is disaggregated by tear fluid and after a few hours fragments separate from the main part and may be expulsed. Therefore, the collagen shield requires a delicate and experienced handling in order not to be adversely applied to the ocular surface. Consequently, this device may be difficult for self–administration by patients.

2.3.2. Liposomes loaded with CsA incorporated in collagen shields

Topical CsA liposomes are able to enhance corneal graft survival [57] but have a too short corneal retention time. Combining the positive features of collagen shields with the advantages of liposomes may provide a synergistic action on the eye. Pleyer [69] showed that such a device was able to significantly improve CsA aqueous humor concentration at 1 and 3 h when compared to CsA loaded liposomes. But, this device did not achieve the aim of prolonging corneal and aqueous humor levels over 6 h. In addition, this system is complex to manufacture and retains the already mentioned drawbacks of the collagen shields.

2.3.3. Collagen particles

To overcome the disadvantages of shields but maintain the benefits of collagen devices, small collagen particles loaded with CsA were manufactured and suspended in a methylcellulose hydrogel. The particle size is relatively large: 2 $mm^2 \pm 0.1$ $mm^2 \times 0.1$ mm. A volume of the suspension containing 0.5 mg CsA was administered on the cornea of rabbits. The particles were more effective than corn oil in delivering CsA to the cornea and anterior chamber. Higher concentrations of CsA were found in both the cornea and aqueous humor of eyes treated with collagen particles. Kinetics of drug penetration were different for drug in the collagen vehicle compared to the corn oil. The particles produced a CsA peak concentration at 4 h and maintained a high level until 8 h (Fig. 5) while corn oil gave a peak at 1 h that almost disappeared at 8 h [70]. This formulation improved significantly corneal allograft survival compared to corn oil [71] but showed no significant improvement compared to the collagen shield. The hydrogel exerts a viscosifying effect and enhances retention time on the cornea. The main advantage of collagen particles is the ease with which the formulation can be applied to the ocular surface and its possibility for self-administration. Whether the sustained release effect is due to the collagen formulation or to the methylcellulose hydrogel remains to be determined. Kinetic studies comparing CsA collagen particles alone and CsA suspended in the methylcellulose hydrogel would be very welcome. Tolerance evaluation is not mentioned and should be evaluated since the particles are quite large.

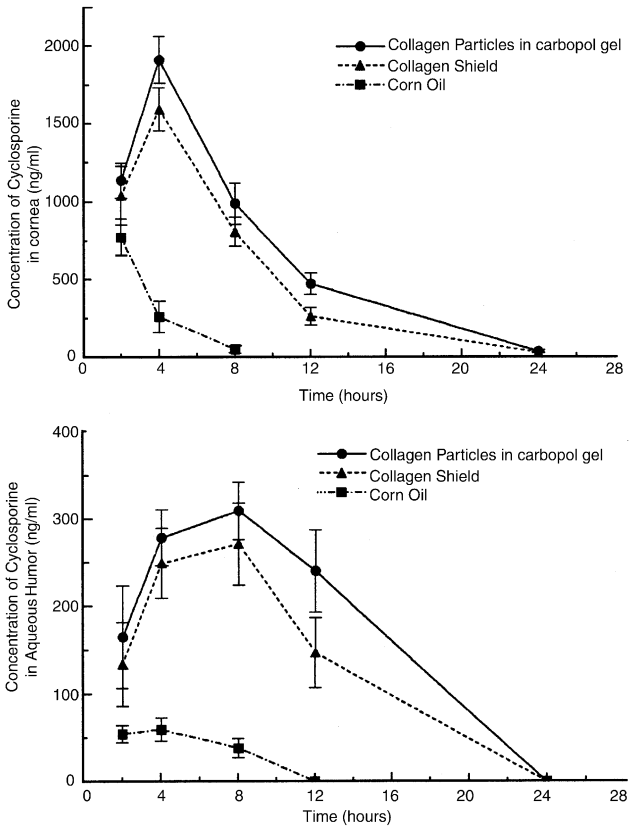

Fig. 5. Concentrations curves of CsA over 24 h in the cornea and aqueous humor after topical application on the rabbit eye of different CsA formulations: collagen particles (■), collagen shield (▲) and corn oil solution (■) [72].

2.4. Chemical modifications of the molecule: prodrug approach

Prodrugs are defined as pharmacologically inactive derivatives of drug that are chemically or enzymatically converted to the active drug. Such molecules are used to modify the physico-chemical properties of the drug or its in vivo behavior. To improve its water solubility, a water-solubilizing moiety was grafted on a free hydroxyl function of CsA [72]. The resulting prodrug could be solubilized in isotonic and neutral solutions at a concentration equivalent to

1% CsA w/v that is approximately 10,000 times the solubility of CsA alone in water. After topical administration of 25 μl of this solution in the rabbit eye, the CsA prodrug is cleaved in vivo by tear enzymes and releases free CsA. This resulted in CsA concentrations in tears above experimental therapeutic levels for up to 20 min. Objective and subjective animal (rabbit) tolerance tests showed an improved tolerance over that of classical CsA-in-oil solution. This prodrug is a promising candidate in the topical treatment of dry eye disease and corneal graft rejection.

Most of the topical delivery systems discussed here did not succeed in achieving aqueous humor therapeutic levels but corneal and conjunctival levels were sufficient to suppress T cell activation. This route will be useful for extraocular diseases. As all devices yielded therapeutic levels, choice between systems will be influenced by the degree of tolerance and ease of administration and manufacture. Altogether, three topical systems are noteworthy: chitosan nanoparticles, positively charged emulsions and CsA prodrugs as they are innovative, well tolerated and result in therapeutic concentrations in extraocular tissues in animal models.

Recently, new non-aqueous solvents were proposed for the topical delivery of lipophilic drugs. Perfluorocarbons or fluorinated silicone liquids are chemically and biologically inert compounds, and present low surface tension, excellent spreading characteristics and close-to-water refractive indices [73]. These solvents could be an alternative to complex topical drug delivery systems.

The insert approach has not yet been proposed to deliver CsA. Inserts are composed of a polymeric support containing a drug. They are usually placed in the lower fornix and may increase contact time between the preparation and the conjunctival tissue to ensure a sustained release. As lipophilic drugs can be incorporated in inserts [74], CsA could be a good candidate for this delivery system.

3. Other administration sites

The other main routes of administration for ocular therapeutics include the subconjunctival, intraocular and systemic pathways. They are employed when drugs are not absorbed by the topical route or when the vitreous humor is the target. We can also briefly mention retrobulbar injection of CsA [75] that was reported to enhance effect on transplant survival, and direct injections of CsA into the anterior chamber [76].

3.1. Subconjunctival administration

The subconjunctival route is an alternative to the topical and intraocular delivery of CsA. Following subconjunctival injections, the administered drug passes through the sclera and into the eye by simple diffusion. Before the injection or implantation, the eye is anaesthetized by a topical local anesthetic. Microspheres and implants have been developed and tested after subconjunctival administration.

3.1.1. Microspheres

In order to maintain high levels of CsA in the cornea and aqueous humor, Harper [77] employed microspheres made of 50:50 D,L-lactide/glycolide copolymer (PLGA) and loaded with CsA. Experimental therapeutic concentrations were reached in the cornea and aqueous humor 6 h after subconjunctival injection, in a rabbit model, of 0.13 ml of the microsphere solution containing a total dose of 2 mg of CsA (approx. 15 mg/ml in the final formulation). These concentrations were maintained for 2 weeks in the cornea after injection. Although these results were encouraging, efficacy tests were not performed on animal models of grafted corneas.

Several years later, the same type of PLGA microspheres were manufactured by another research group with a 15 mg/ml CsA concentration in the final formulation and was tested on keratoplasty rejection in the rabbit [78]. The mean survival time of the graft eye treated by CsA loaded microspheres was 117 days. The formulation did not show significant differences to CsA oil solution with respect to survival time (Fig. 6) but histopathology showed improved local tolerance.

Thus, although these two studies used the same polymer, tissues concentrations were different. Despite a higher CsA amount injected (3 mg), Rojas Silva observed lower corneal and aqueous CsA levels compared to the study that injected 2 mg of CsA in the formulation [77]. This disparity could be explained by a difference in the manufacturing protocols or by the fact that the two studies used different strains of rabbits.

3.1.2. Subconjunctival injection of liposomes

Liposomes, already mentioned as potential topical drug carriers, also have an application in subconjunctival

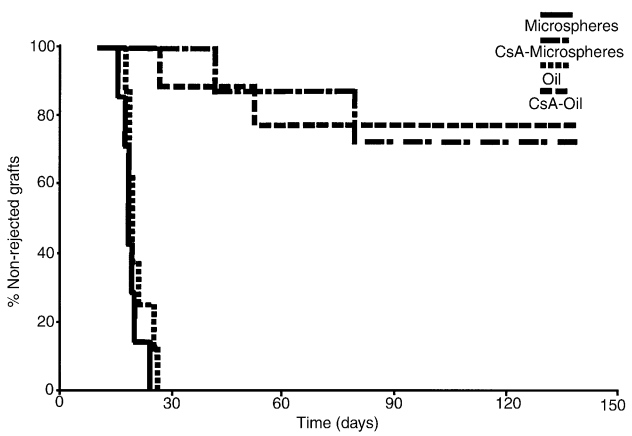

Fig. 6. Percentage of non-rejected grafted corneas after topical treatment with empty PLGA microspheres (—), CsA-loaded PLGA microspheres (— -), castor oil (- - - -) and CsA in castor oil (– – –) on a rat model over 150 days [79].

injection. A CsA-loaded liposome suspension was injected subconjunctivally [79] and the concentrations of CsA, in the aqueous and vitreous humor of albino rabbits were measured, at intervals of 24, 48, 96, and 192 h. The aqueous humor concentration of CsA was high until day 4 in the group receiving liposomes and in the group receiving free CsA (1438 and 1050 ng/ml, respectively). The vitreous concentration was very low in both groups. However, liposomes did not show significant improvement in terms of tissue concentrations compared to free CsA.

3.1.3. Subconjunctival implant

Degradable polylactide-co-glycolide (PLGA) copolymers of composition 85:15 lactide/glycolide were used to manufacture CsA loaded implants in a disk shape of 5 mm in diameter and 0.5 mm thick [80]. Implants were sterilized by γ-irradiation and could contain up to 50% w/w CsA. This biodegradable system showed a high and constant release of CsA over 15 days in vitro at 37 °C in a balanced saline solution. Implants were surgically placed in direct contact with the sclera after the subconjunctival space was opened (rabbit model). The CsA loaded implants improved corneal graft survival from 18 ± 4 days with the placebo to 35 ± 7 days. This improvement in survival time by 15 days corresponds to the 15 days of CsA in vitro release from the implant. No systemic CsA was detected, suggesting that the effect was local. Histological assessment indicated that devices were well tolerated.

The advantage of this approach is that it may provide therapeutic levels of CsA during approximately 15 days in the extraocular area and its major drawback is its invasiveness. However, the subconjunctival route is very useful when aqueous humor is the target and solid forms should allow a better controlled release.

3.2. Intraocular delivery

Intravitreal drug injection is the usual route for posterior segment disorder therapy. It consists of the injection or implantation of the drug formulation in the aqueous or vitreous humor. Histological retinal changes were found with intravitreal injections of 200 μg of CsA [81] but no histological or electroretinographic changes were observed with doses of 100 μg or less. This non-toxic dose is projected to be at least 77 times the amount needed to be effective.

As CsA does not induce a toxic effect on the retina, the drug can be directly injected in the vitreous [82]. This procedure involves a high risk of complications especially when repeated injections are required. To circumvent this problem, new sustained release systems were proposed for CsA intraocular delivery.

3.2.1. Intravitreal injection of liposome-bound CsA

High CsA levels in the vitreous are required in the treatment of severe posterior uveitis. To achieve this goal and maintain therapeutic levels long enough, liposome-bound CsA was injected intravitreally. Alghadyan [83] demonstrated that liposome-bound CsA was safe up to the dose of 500 μg CsA. Then 100 μg of CsA bound to liposomes was injected into the posterior chamber of rabbits. This system provided high concentrations over 8 days compared to the injection of free CsA that was undetectable at 8 days [84]. Liposomes showed sustained release of CsA but this system needs further investigation to prove therapeutic efficacy.

3.2.2. Biodegradable implants

Small PLGA implants [85] (cylinder of 2-mm diameter and 1.5-mm length, loaded with 0.3 mg CsA) were implanted in the anterior chamber of corneal grafted rabbits. The implants resulted in a 17-day graft survival while the 1% w/v CsA drops treated eyes showed a 10-day graft survival. Besides a mild inflammation after 4 days, the treated eyes remained normal at histopathologic examinations. Therapeutic levels were maintained over 2 weeks in the aqueous humor. The advantage of this system is that the implant is placed directly in the anterior chamber after the ablation of the cornea. The implant is degradable and there is no need for further surgery to remove the system. However, the half-life of CsA in the aqueous humor is too short. Further investigations are needed to determine precisely where CsA is released in the eye.

3.2.3. Non-biodegradable implants

Vitrasert® is a surgically implantable device for sustained intravitreal release. The device delivers ganciclovir intraocularly over approximately 4–5 months to treat cytomegalovirus retinitis [86]. This system has been extended to CsA delivery [87]. A small quantity of CsA is compressed in a tablet die to make a pellet. Each pellet is coated with polyvinyl alcohol, covered on the sides by ethylene vinyl acetate and heated for 1 h. The duration and temperature of heat treatment can be varied to control the release rate. Implants are surgically placed and sutured to the conjunctiva with a polyvinyl alcohol strip. The in vivo release rate is 1.3 mg/day and a concentration of 500 ng/ml is achieved in the vitreous, which is approximately five times the therapeutic concentration, and this over a 6-month period [87]. The intravitreal CsA device effectively suppressed ocular inflammation in a rabbit model of uveitis [88]. This device is now under a phase I/II clinical trial at the National Eye Institute and Duke University for the treatment of uveitis [89]. Dexamethasone can be added to CsA in the implant to enhance efficacy as both molecules reduce inflammation by different mechanisms [90].

A veterinary application of the concept is being evaluated on experimental uveitis in horses. The implants are well tolerated with no long term complications [91] and showed beneficial effects on intraocular inflammation [92]. However, the Vitrasert® implant was withdrawn from the human European market in April 2002 by the European

Agency for Evaluation of Medicinal Products because of ocular complications (retinal detachment).

3.3. Intravenous injection

Systemic administration of CsA was shown to be beneficial in inflammatory ocular diseases [93]. A study [94] assessed the effect of systemic CsA treatment on graft rejection on a rat model. CsA prevented rejection as long as treatment was given but occurred in most grafts within 10 days of treatment cessation. Nevertheless, systemic side effects limit the usefulness of systemic administration.

4. Conclusion

Pharmaceutical technology has been shown to successfully improve some of the unfavorable physico-chemical properties of CsA by enhancing ocular availability and improving tolerance. Considering topical delivery, chitosan nanoparticles, positively charged emulsions and CsA prodrugs seem to be the most promising candidates. However, none of the described topical systems has really succeeded in achieving therapeutic concentrations for an extended period of time on the corneal surface. Furthermore, these formulations were not able to achieve CsA concentrations in the aqueous humor and in the vitreous. Intraocular target can only be reached by i.v. injection, intravitreal injection or device implantation. At the present time, despite the need for surgery, the non-biodegradable implant (Vitrasert® type) is the most promising device for intraocular delivery but the ideal system would be biodegradable implants.

The problem of local delivery of CsA is not only a problem of formulation but also that of a lack of medical knowledge. Several points, such as mechanisms of action, targets and therapeutic dose must be more clearly identified and understood.

In the treatment of dry eye syndrome, immunosuppression may not be the only mechanism of action as

Table 1
Main delivery systems developed for CsA delivery to the eye

Pathway	Dosage forms	Delivery systems	Advantages	Drawbacks	References
Topical	Solutions	Oils	High solubilizing CsA capacity	Poor tolerance Unfavorable partition	[7]
		α-cyclodextrins	Enhanced corneal penetration	Repeated administrations	[39,40]
		Penetration enhancers	Enhanced corneal penetration	Poor tolerance	[43,41]
	Colloidal carriers	Micelles	High corneal concentrations at 24 hours	Stability of micelles	[49]
		Emulsion negatively-charged	Improvement in dry eye symptoms, FDA approved	Ocular burning	[54,24]
		Emulsion positively-charged	Enhancement of corneal retention time, high levels in cornea and conjunctiva	Tolerance to be evaluated	[57]
		Liposomes	Good tolerance, improved survival time of corneal graft	Short retention time on cornea	[58]
		PCL nanoparticles	Enhanced extraocular retention time, high corneal uptake	No effect on corneal grafts	[60]
		PACA nanoparticles	Improved corneal absorption	Poor tolerance	[64]
		Chitosan nanoparticles	Good tolerance, high extraocular concentrations	Natural origin of chitosan	[66]
	Solid forms	Collagen shields	Bandage effect, high levels in cornea	Patient discomfort No self-administration	[69]
		Collagen shields + liposomes	Slow continuous release, high tissue concentrations	Complexity of manufacture	[70]
		Collagen particles	Effective in the prevention of graft cornea rejection	No significant improvement in drug concentration	[71]
	Chemical modifications	Prodrugs	Good tolerance, soluble in water, high tear concentrations	Aqueous stability	[73]
Subconjunctival	Liquids	Microspheres	High levels in cornea and aqueous humor	No prevention of graft rejection	[78,79]
		Liposomes	High levels at four days in aqueous humor	No benefits compared to free CsA	[80]
	Solid forms	Biodegradable implants	15 day improvement in the prevention of graft cornea rejection	Surgery for implantation	[81]
Intraocular	Liquids	Liposomes	Three day half life in the vitreous	Frequency of injection	[85]
	Solid forms	Biodegradable implants	Four week therapeutics levels in vitreous, prolongation of corneal graft survival	Implant is free in the anterior chamber	[86]
		Non-biodegradable implants	Controlled release, effective over several months	Removal surgery	[88]

demonstrated by Palmer [95] who showed that systemic CsA could induce lachrymation in patients with a normal baseline tear production.

Topical administration is, obviously, the main route for the treatment of surface symptoms and diseases, such as Sjörgren syndrome or vernal keratoconjunctivitis, or more precisely, when the targeted organs are the cornea, conjunctiva, lachrymal gland or local drainage system. But, in the case of corneal graft, epithelial rejections might be controlled by topical administration of CsA while endothelial rejections would probably not be controlled by local application.

Treatment of intraocular diseases like uveitis or other intraocular inflammation, require high CsA concentrations in the aqueous humor and the iris/ciliary body. It is not clear, however, whether or not significant systemic levels of drug are needed to block cell-mediated immune response in the regional ganglions to obtain an immunosuppressive effect in the eye.

At the present time, only one ophthalmic formulation of CsA is commercially available (Restasis®) and the extensive literature on the delivery of CsA (Table 1) to the eye reflects the great medical interest and the pharmacoeconomical aspects of this challenge.

References

[1] S. Noble, A. Markham, Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral), Drugs 50 (1995) 924–941.

[2] R.B. Nussenblatt, A.G. Palestine, C.C. Chan, G. Stevens, S.D. Mellow, S.B. Green, Randomized, double-masked study of cyclosporine compared to prednisolone in the treatment of endogenous uveitis, Am. J. Ophthalmol. 112 (1991) 138–146.

[3] K. Masuda, A. Nakajima, A. Urayama, K. Nakae, M. Kogure, G. Inaba, Double-masked trial of cyclosporin versus colchicine and long-term open study of cyclosporin in Behcet's disease, Lancet 1 (1989) 1093–1096.

[4] C. Georganas, D. Ioakimidis, C. Iatrou, B. Vidalaki, K. Iliadou, P. Athanassiou, T. Kontomerkos, Relapsing Wegener's granulomatosis: successful treatment with cyclosporin-A, Clin. Rheumatol. 15 (1996) 189–192.

[5] M.F. Prummel, M.P. Mourits, A. Berghout, E.P. Krenning, R. van der Gaag, L. Koornneef, W.M. Wiersinga, Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy, N. Engl. J. Med. 321 (1989) 1353–1359.

[6] T. Reinhard, R. Sundmacher, E. Godehardt, P. Heering, Preventive systemic cyclosporin A after keratoplasty at increased risk for immune reactions as the only elevated risk factor, Ophthalmologe 94 (1997) 496–500.

[7] D. BenEzra, G. Maftzir, C. de Courten, P. Timonen, Ocular penetration of cyclosporin A. III: The human eye, Br. J. Ophthalmol. 74 (1990) 350–352.

[8] M.J. Mihatsch, M. Kyo, K. Morozumi, Y. Yamaguchi, V. Nickeleit, B. Ryffel, The side-effects of ciclosporine-A and tacrolimus, Clin. Nephrol. 49 (1998) 356–363.

[9] C. Li, C.W. Yang, H.J. Ahn, W.Y. Kim, C.W. Park, J.H. Park, J.H. Cha, J. Kim, Y.S. Kim, B.K. Bang, Colchicine suppresses osteopontin expression and inflammatory cell infiltration in chronic cyclosporine nephrotoxicity, Nephron 92 (2002) 422–430.

[10] V. Ganesan, D.V. Milford, C.M. Taylor, S.A. Hulton, S. Parvaresh, P. Ramani, Cyclosporin-related nephrotoxicity in children with nephrotic syndrome, Pediatr. Nephrol. 17 (2002) 225–226.

[11] V. Gupta, P.K. Sahu, Topical cyclosporin A in the management of vernal keratoconjunctivitis, Eye 15 (2001) 39–41.

[12] K.S. Kunert, A.S. Tisdale, I.K. Gipson, Goblet cell numbers and epithelial proliferation in the conjunctiva of patients with dry eye syndrome treated with cyclosporine, Arch. Ophthalmol. 120 (2002) 330–337.

[13] H.D. Perry, S.J. Doshi, E.D. Donnenfeld, G.S. Bai, Topical cyclosporin A in the management of therapeutic keratoplasty for mycotic keratitis, Cornea 21 (2002) 161–163.

[14] L.D. Bowers, D.M. Canafax, Cyclosporine: experience with therapeutic monitoring, Ther. Drug Monit. 6 (1984) 142–147.

[15] N. el Tayar, A.E. Mark, P. Vallat, R.M. Brunne, B. Testa, W.F. van Gunsteren, Solvent-dependent conformation and hydrogen-bonding capacity of cyclosporin A: evidence from partition coefficients and molecular dynamics simulations, J. Med. Chem. 36 (1993) 3757–3764.

[16] S.D. Mithani, V. Bakatselou, C.N. TenHoor, J.B. Dressman, Estimation of the increase in solubility of drugs as a function of bile salt concentration, Pharm. Res. 13 (1996) 163–167.

[17] D. BenEzra, G. Maftzir, Ocular penetration of cyclosporin A. The rabbit eye, Invest. Ophthalmol. Vis. Sci. 31 (1990) 1362–1366.

[18] A.M. el Asrar, K.F. Tabbara, K. Geboes, L. Missotten, V. Desmet, An immunohistochemical study of topical cyclosporine in vernal keratoconjunctivitis, Am. J. Ophthalmol. 121 (1996) 156–161.

[19] D.K. Olivero, M.G. Davidson, R.V. English, M.P. Nasisse, V.E. Jamieson, T.M. Gerig, Clinical evaluation of 1% cyclosporine for topical treatment of keratoconjunctivitis sicca in dogs, J. Am. Vet. Med. Assoc. 199 (1991) 1039–1042.

[20] J.C. Zhao, X.Y. Jin, Local therapy of corneal allograft rejection with cyclosporine, Am. J. Ophthalmol. 119 (1995) 189–194.

[21] E.J. Holland, T.W. Olsen, J.M. Ketcham, C. Florine, J.H. Krachmer, J.J. Purcell, S. Lam, H.H. Tessler, J. Sugar, Topical cyclosporin A in the treatment of anterior segment inflammatory disease, Cornea 12 (1993) 413–419.

[22] K. Gunduz, O. Ozdemir, Topical cyclosporin treatment of keratoconjunctivitis sicca in secondary Sjogren's syndrome, Acta Ophthalmol. (Copenh) 72 (1994) 438–442.

[23] D. Stevenson, J. Tauber, B.L. Reis, Efficacy and safety of cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. The Cyclosporin A Phase 2 Study Group, Ophthalmology 107 (2000) 967–974.

[24] R.L. Kaswan, Intraocular penetration of topically applied cyclosporine, Transpl. Proc. 20 (1988) 650–655.

[25] D.L. Williams, A comparative approach to topical cyclosporine therapy, Eye 11 (1997) 453–464.

[26] S. Solch, P.I. Nadler, M.H. Silverman, Safety and tolerability of two percent cyclosporine (Sandimmune) ophthalmic ointment in normal volunteers, J. Ocul. Pharmacol. 7 (1991) 301–312.

[27] S.K. Knagenhjelm, K. Froyland, A. Ringvold, E. Bjerkas, I. Kjonniksen, Toxicological evaluation of cyclosporine eyedrops, Acta Ophthalmol. Scand. 77 (1999) 200–203.

[28] S. Kachi, K. Hirano, Y. Takesue, M. Miura, Unusual corneal deposit after the topical use of cyclosporine as eyedrops, Am. J. Ophthalmol. 130 (2000) 667–669.

[29] M. Sznitowska, K. Zurowska-Pryczkowska, E. Dabrowska, S. Janicki, Increased partitioning of pilocarpine to the oily phase of submicron emulsion does not result in improved ocular bioavailability, Int. J. Pharm. 202 (2000) 161–164.

[30] M. Wiederholt, D. Kossendrup, W. Schulz, F. Hoffmann, Pharmacokinetic of topical cyclosporin A in the rabbit eye, Invest. Ophthalmol. Vis. Sci. 27 (1986) 519–524.

[31] A. Heiligenhaus, K.P. Steuhl, Treatment of HSV-1 stromal keratitis with topical cyclosporin A: a pilot study, Graefes Arch. Clin. Exp. Ophthalmol. 237 (1999) 435–438.
[32] R.V. Morgan, K.L. Abrams, Topical administration of cyclosporine for treatment of keratoconjunctivitis sicca in dogs, J. Am. Vet. Med. Assoc. 199 (1991) 1043–1046.
[33] L. Cheeks, R.L. Kaswan, K. Green, Influence of vehicle and anterior chamber protein concentration on cyclosporine penetration through the isolated rabbit cornea, Curr. Eye Res. 11 (1992) 641–649.
[34] M.W. Mosteller, B.M. Gebhardt, A.M. Hamilton, H.E. Kaufman, Penetration of topical cyclosporine into the rabbit cornea, aqueous humor, and serum, Arch. Ophthalmol. 103 (1985) 101–102.
[35] M. Diaz-Llopis, J.L. Menezo, Penetration of 2% cyclosporin eyedrops into human aqueous humour, Br. J. Ophthalmol. 73 (1989) 600–603.
[36] D.W. Mueller, Improved extemporaneous formulation of cyclosporine ophthalmic drops, Am. J. Hosp. Pharm. 51 (1994) 3080–3081.
[37] J. Sansom, K.C. Barnett, W. Neumann, A. Schulte-Neumann, B. Clerc, J.P. Jegou, V. Haas, A. Weingarten, Treatment of keratoconjunctivitis sicca in dogs with cyclosporine ophthalmic ointment: a European clinical field trial. Vet. Rec. 137 (1995) 504–507.
[38] T. Loftsson, E. Stefansson, Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye, Acta Ophthalmol. Scand. 80 (2002) 144–150.
[39] A. Kanai, R.M. Alba, T. Takano, C. Kobayashi, A. Nakajima, K. Kurihara, T. Yokoyama, M. Fukami, The effect on the cornea of alpha cyclodextrin vehicle for cyclosporin eye drops, Transpl. Proc. 21 (1989) 3150–3152.
[40] C. Newton, B.M. Gebhardt, H.E. Kaufman, Topically applied cyclosporine in azone prolongs corneal allograft survival, Invest Ophthalmol. Vis. Sci. 29 (1988) 208–215.
[41] D.D. Tang-Liu, J.B. Richman, R.J. Weinkam, H. Takruri, Effects of four penetration enhancers on corneal permeability of drugs in vitro, J. Pharm. Sci. 83 (1994) 85–90.
[42] P. van der Bijl, A.D. van Eyk, D. Meyer, Effects of three penetration enhancers on transcorneal permeation of cyclosporine, Cornea 20 (2001) 505–508.
[43] P. Furrer, J.M. Mayer, B. Plazonnet, R. Gurny, Ocular tolerance of preservatives on the murine cornea, Eur. J. Pharm. Biopharm. 47 (1999) 105–112.
[44] E. Adriaens, K. Dierckens, T.G. Bauters, H.J. Nelis, F. van Goethem, P. Vanparys, J.P. Remon, The mucosal toxicity of different benzalkonium chloride analogues evaluated with an alternative test using slugs, Pharm. Res. 18 (2001) 937–942.
[45] D. Monti, P. Chetoni, S. Burgalassi, M. Najarro, M.F. Saettone, Increased corneal hydration induced by potential ocular penetration enhancers: assessment by differential scanning calorimetry (DSC) and by desiccation, Int. J. Pharm. 232 (2002) 139–147.
[46] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, E.L. Cremophor, The drawbacks and advantages of vehicle selection for drug formulation, Eur. J. Canc. 37 (2001) 1590–1598.
[47] P. Calvo, M.J. Alonso, J.L. Vila-Jato, J.R. Robinson, Improved ocular bioavailability of indomethacin by novel ocular drug carriers, J. Pharm. Pharmacol. 48 (1996) 1147–1152.
[48] M. Kuwano, H. Ibuki, N. Morikawa, A. Ota, Y. Kawashima, Cyclosporine A formulation affects its ocular distribution in rabbits, Pharm. Res. 19 (2002) 108–111.
[49] S. Hickok, Polyoxyethylene stearate, in: A. Wade, P. Weller (Eds.), Handbook of Pharmaceutical Excipients, The Pharmaceutical Press, London, UK, 1994, pp. 379–384.
[50] S. Muchtar, S. Almog, M.T. Torracca, M.F. Saettone, S. Benita, A submicron emulsion as ocular vehicle for delta-8- tetrahydrocannabinol: effect on intraocular pressure in rabbits, Ophthalmic Res. 24 (1992) 142–149.
[51] N. Naveh, S. Muchtar, S. Benita, Pilocarpine incorporated into a submicron emulsion vehicle causes an unexpectedly prolonged ocular hypotensive effect in rabbits, J. Ocul. Pharmacol. 10 (1994) 509–520.
[52] S. Ding. Non-irritating emulsions for sensitive tissue, U.S. Patent 5, 474,979, 1995.
[53] A.A. Acheampong, M. Shackleton, D.D. Tang-Liu, S. Ding, M.E. Stern, R. Decker, Distribution of cyclosporin A in ocular tissues after topical administration to albino rabbits and beagle dogs, Curr. Eye Res. 18 (1999) 91–103.
[54] K. Sall, O.D. Stevenson, T.K. Mundorf, B.L. Reis, Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 3 Study Group [published erratum appears in Ophthalmology 2000 Jul;107(7):1220.], Ophthalmology 107 (2000) (2000) 631–639.
[55] S. Klang, M. Abdulrazik, S. Benita, Influence of emulsion droplet surface charge on indomethacin ocular tissue distribution, Pharm. Dev. Technol. 5 (2000) 521–532.
[56] M. Abdulrazik, S. Tamilvanan, K. Khoury, S. Benita, Ocular delivery of cyclosporine A. Effect of submicron emulsion's surface charge on ocular distribution of topical cyclosporin A, S. T. P. Pharma Sci. 11 (2001) 427–432.
[57] J.K. Milani, U. Pleyer, A. Dukes, H.J. Chou, S. Lutz, D. Ruckert, K.H. Schmidt, B.J. Mondino, Prolongation of corneal allograft survival with liposome-encapsulated cyclosporine in the rat eye, Ophthalmology 100 (1993) 890–896.
[58] S.L. Law, K.J. Huang, C.H. Chiang, Acyclovir-containing liposomes for potential ocular delivery. Corneal penetration and absorption, J. Controlled Rel. 63 (2000) 135–140.
[59] P. Calvo, A. Sanchez, J. Martinez, M.I. Lopez, M. Calonge, J.C. Pastor, M.J. Alonso, Polyester nanocapsules as new topical ocular delivery systems for cyclosporin A, Pharm. Res. 13 (1996) 311–315.
[60] P. Calvo, C. Thomas, M.J. Alonso, J.L. Vila-Jato, J.R. Robinson, Study of the mechanism of interaction of poly(E-caprolactone) nanocapsules with the cornea by confocal laser scanning microscopy, Int. J. Pharm. 103 (1994) 283–291.
[61] J.R. Juberias, M. Calonge, S. Gomez, M.I. Lopez, P. Calvo, J.M. Herreras, M.J. Alonso, Efficacy of topical cyclosporine-loaded nanocapsules on keratoplasty rejection in the rat, Curr. Eye Res. 17 (2002) 39–46.
[62] V. Masson, F. Maurin, H. Fessi, J.P. Devissaguet, Influence of sterilization processes on poly(E-caprolactone) nanospheres, Biomaterials 18 (1997) 327–335.
[63] C.A. Le Bourlais, F. Chevanne, B. Turlin, L. Acar, H. Zia, P.A. Sado, T.E. Needham, R. Leverge, Effect of cyclosporine A formulations on bovine corneal absorption: ex-vivo study, J. Microencapsul. 14 (1997) 457–467.
[64] A. Zimmer, J. Kreuter, J.R. Robinson, Studies on the transport pathway of PBCA nanoparticles in ocular tissues, J. Microencapsul. 8 (1991) 497–504.
[65] A.M. De Campos, A. Sanchez, M.J. Alonso, Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A, Int. J. Pharm. 224 (2001) 159–168.
[66] O. Felt, P. Furrer, J.M. Mayer, B. Plazonnet, P. Buri, R. Gurny, Topical use of chitosan in ophthalmology: tolerance assessment and evaluation of precorneal retention, Int. J. Pharm. 180 (1999) 185–193.
[67] L.Y. Lim, E. Khor, C.E. Ling, Effects of dry heat and saturated steam on the physical properties of chitosan, J. Biomed. Mater. Res. 48 (1999) 111–116.
[68] J.J. Reidy, B.M. Gebhardt, H.E. Kaufman, The collagen shield. A new vehicle for delivery of cyclosporin A to the eye, Cornea 9 (1990) 196–199.
[69] U. Pleyer, B. Elkins, D. Ruckert, S. Lutz, J. Grammer, J. Chou, K.H. Schmidt, B.J. Mondino, Ocular absorption of cyclosporine A from liposomes incorporated into collagen shields, Curr. Eye Res. 13 (1994) 177–181.
[70] B.M. Gebhardt, H.E. Kaufman, Collagen as a delivery system for hydrophobic drugs: studies with cyclosporine, J. Ocul. Pharmacol. Ther. 11 (1995) 319–327.

[71] B.M. Gebhardt, E.D. Varnell, H.E. Kaufman, Cyclosporine in collagen particles: corneal penetration and suppression of allograft rejection, J. Ocul. Pharmacol. Ther. 11 (1995) 509–517.
[72] R. Wenger, M. Mutter, A. Hamel, F. Hubler. Pro-Drug and use thereof as a medicament, WO Patent 02/085928, 2002.
[73] Meadows D. Nonaqueous fluorinated drug delivery vehicle suspensions, U.S. Patent 5,173,298, 1992.
[74] S. Kawakami, K. Nishida, T. Mukai, K. Yamamura, J. Nakamura, T. Sakaeda, M. Nakashima, H. Sasaki, Controlled release and ocular absorption of tilisolol utilizing ophthalmic insert-incorporated lipophilic prodrugs, J. Controlled Rel. 76 (2001) 255–263.
[75] J.D. Salisbury, B.M. Gebhardt, Suppression of corneal allograft rejection by cyclosporin A, Arch. Ophthalmol. 99 (1981) 1640–1643.
[76] C. Oh, A.J. Apel, B.A. Saville, Y.L. Cheng, D.S. Rootman, Local efficacy of cyclosporine in corneal transplant therapy, Curr. Eye Res. 13 (1994) 337–343.
[77] C.A. Harper, B. Khoobehi, G.A. Peyman, B.M. Gebhardt, W.A. Dunlap, Bioavailability of microsphere-entrapped cyclosporine A in the cornea and aqueous of rabbits, Int. Ophthalmol. 17 (1993) 337–340.
[78] M.V. Rojas Silva, M.T. Rodriguez-Ares, M. Sanchez-Salorio, M.J. Lamas Diaz, A.J. Cuevas, J.L. Vila Jato, T.C. Capeans, Efficacy of subconjunctival cyclosporin-containing microspheres on keratoplasty rejection in the rabbit, Graefes Arch. Clin. Exp. Ophthalmol. 237 (1999) 840–847.
[79] A.A. Alghadyan, G.A. Peyman, B. Khoobehi, S. Milner, K.R. Liu, Liposome-bound cyclosporine: aqueous and vitreous level after subconjunctival injection, Int. Ophthalmol. 12 (1988) 101–104.
[80] A. Apel, C. Oh, R. Chiu, B. Saville, Y.L. Cheng, D. Rootman, A subconjunctivalde gradable implant for cyclosporine delivery in corneal transplant therapy, Curr. Eye Res. 14 (1995) 659–667.
[81] J. Grisolano, G.A. Peyman, Retinal toxicity study of intravitreal cyclosporin, Ophthalmic Surg. 17 (1986) 155–156.
[82] C.C. Lai, P. Gouras, K. Doi, S.H. Tsang, S.P. Goff, P. Ashton, Local immunosuppression prolongs survival of RPE xenografts labeled by retroviral gene transfer, Invest. Ophthalmol. Vis. Sci. 41 (2000) 3134–3141.
[83] A.A. Alghadyan, G.A. Peyman, B. Khoobehi, K.R. Liu, Liposome-bound cyclosporine: retinal toxicity after intravitreal injection, Int. Ophthalmol. 12 (1988) 105–107.
[84] A.A. Alghadyan, G.A. Peyman, B. Khoobehi, S. Milner, K.R. Liu, Liposome-bound cyclosporine: clearance after intravitreal injection, Int. Ophthalmol. 12 (1988) 109–112.
[85] L. Xie, W. Shi, Z. Wang, J. Bei, S. Wang, Prolongation of corneal allograft survival using cyclosporine in a polylactide-co-glycolide polymer, Cornea 20 (2001) 748–752.
[86] G.E. Sanborn, R. Anand, R.E. Torti, S.D. Nightingale, S.X. Cal, B. Yates, P. Ashton, T. Smith, Sustained-release ganciclovir therapy for treatment of cytomegalovirus retinitis. Use of an intravitreal device, Arch. Ophthalmol. 110 (1992) 188–195.
[87] P.A. Pearson, G.J. Jaffe, D.F. Martin, G.J. Cordahi, H. Grossniklaus, E.T. Schmeisser, P. Ashton, Evaluation of a delivery system providing long-term release of cyclosporine, Arch. Ophthalmol. 114 (1996) 311–317.
[88] G.J. Jaffe, C.S. Yang, X.C. Wang, S.W. Cousins, R.P. Gallemore, P. Ashton, Intravitreal sustained-release cyclosporine in the treatment of experimental uveitis, Ophthalmology 105 (1998) 46–56.
[89] G. Velez, S.M. Whitcup, New developments in sustained release drug delivery for the treatment of intraocular disease, Br. J. Ophthalmol. 83 (1999) 1225–1229.
[90] L.B. Enyedi, P.A. Pearson, P. Ashton, G.J. Jaffe, An intravitreal device providing sustained release of cyclosporine and dexamethasone, Curr. Eye Res. 15 (1996) 549–557.
[91] B.C. Gilger, E. Malok, T. Stewart, P. Ashton, T. Smith, G.J. Jaffe, J.B. Allen, Long-term effect on the equine eye of an intravitreal device used for sustained release of cyclosporine A, Vet. Ophthalmol. 3 (2000) 105–110.
[92] B.C. Gilger, E. Malok, T. Stewart, D. Horohov, P. Ashton, T. Smith, G.J. Jaffe, J.B. Allen, Effect of an intravitreal cyclosporine implant on experimental uveitis in horses, Vet. Immunol. Immunopathol. 76 (2000) 239–255.
[93] D. Wakefield, P. McCluskey, Cyclosporine: a therapy in inflammatory eye disease, J. Ocul. Pharmacol. 7 (1991) 221–226.
[94] C.P. Herbort, M. Matsubara, M. Nishi, M. Mochizuki, Penetrating keratoplasty in the rat: a model for the study of immunosuppressive treatment of graft rejection, Jpn. J. Ophthalmol. 33 (1989) 212–220.
[95] S.L. Palmer, P.A. Bowen, K. Green, Longitudinal tear study after cyclosporine in kidney transplant recipients, Ophthalmology 103 (1996) 670–673.