Expert Opinion

Advanced delivery of Ciclosporin A: present state and perspective

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Ciclosporin A has been used as an immunosuppressor for organ transplantation and other autoimmune disorders for a number of years. Its poor biopharmaceutical characteristics of low solubility and permeability makes the uphill task of designing delivery systems even more challenging for the drug delivery scientist. Works have been performed to investigate administration through various body routes, and have employed approaches that use as emulsions, microspheres, nanoparticles, liposomes, physical and chemical penetration enhancers. Although progress has been made, there is still room for improvement in the application of ciclosporin A, as none of these formulations is ideal.

Keywords: ciclosporin A, dermal delivery, ocular delivery, oral delivery, parenteral delivery, pulmonary delivery

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1. Introduction

The discovery of ciclosporin A (CsA) is one of the greatest discoveries in the history of organ transplantation in the last few decades. CsA was first isolated from a crude extract of the fungus *Tolypocladium inflatum gams* by Sandoz in 1971 [1]. In November 1983, the FDA approved ciclosporin for the prevention of transplant rejection. Wenger reported the complete chemical synthesis of ciclosporin in 1984 [2].

CsA effectively suppresses a T cell-dependent immune reaction. It is well established that CsA, through the formation of a complex with cyclophilin, inhibits the phosphatase activity of calcineurin, which regulates nuclear translocation and the subsequent activation of the nuclear factor of activated T cells transcription factors [3]. Therefore, most of the diseases that involve cytokines or immune-related disorders are potential targets of CsA.

CsA is a highly lipophilic neutral cyclic peptide consisting of 11 amino acids, 7 of which are *N*-methylated. Its molecular formula is $C_{12}H_{111}N_{11}O_{12}$ and its molecular weight is 1202.64 Da. It contains four intra-molecular hydrogen bonds, which impart high rigidity to its cyclic structure (**Figure 1**) [4]. This unusual structural property confers a very low aqueous solubility to this drug. The low water solubility of the drug is a serious problem, causing highly variable and incomplete absorption from its conventional oral or topical formulations, which leads to a tremendous variation in drug pharmacokinetics and, consequently, to an uncertain relation between the drug dosage and *in vivo* exposure.

Another drawback to CsA is the need for careful blood-level monitoring, due to its narrow therapeutic range and variable absorption characteristics [5]. Today, a limited sampling strategy (2 h postdose) shows great promise as a comparatively simple, safe and effective method to optimise patient outcomes during both short-term and maintenance CsA therapy. However, it is not clear whether this method is useful for the optimisation of treatment with generic formulations of CsA [6].

The most common and potentially serious side effects induced by CsA are high blood pressure and kidney problems. Approximately 25% of patients taking CsA for rheumatoid arthritis develop mild-to-moderate high blood pressure. Approximately 50% of patients develop mild kidney problems while on this medication, and may

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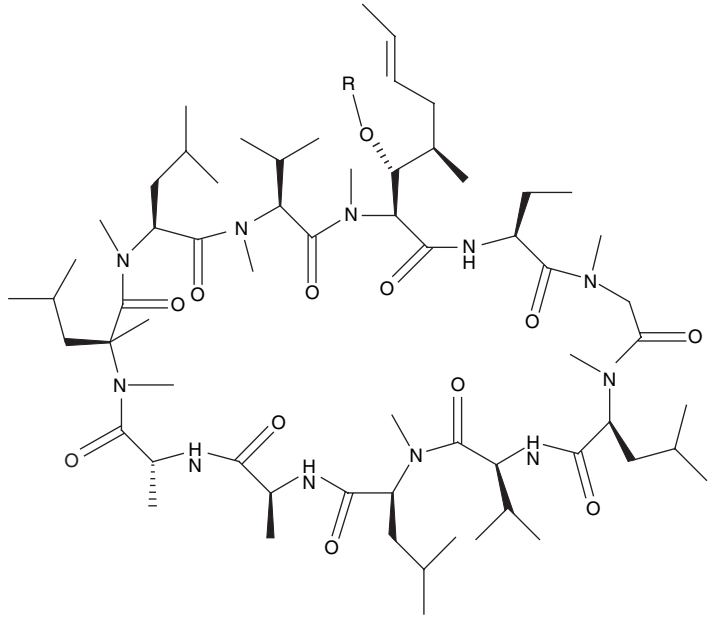

Figure 1. The structure of Ciclosporin A.

need to adjust their dosage or discontinue the medication. Although CsA-induced nephrotoxicity is generally reversible, it can cause irreversible structural changes such as interstitial fibrosis. Therefore, close monitoring of the parameters assessing renal function is required. Other commonly experienced side effects include headaches, nausea, vomiting, abdominal pain or dyspepsia, and swelling of the hands or feet [7,8].

Many research efforts have been made to overcome the abovementioned difficulties and to increase the therapeutic efficacy of CsA, and to decrease its side effects by various body routes employing approaches such as solid formulations, liposomes, emulsions and microemulsions, microspheres, nanoparticles and physical or chemical enhancers (**Table 1**). This review summarises the main pharmaceutical systems and devices that have been described for systemic and topical delivery of CsA.

2. Newer delivery approaches for ciclosporin A

2.1 Oral delivery systems

This is the most preferred route, and most of the drugs available today are in the form of tablets or capsules. However, the problem with CsA administration via the oral route is its low absorption through the gastrointestinal tract [9,10]. A number of different reasons have been suggested for the low and erratic systemic exposure upon oral administration. For example, CsA is prone to extensive gut wall and hepatic metabolism [9], with cytochrome P450 3A being the major enzyme involved in this process [11]. Additionally, the efflux transporter P-glycoprotein seems to play a major role in the inter-subject variability in the oral bioavailability of CsA [12,13]. The small intestine, where CsA has been found to be predominantly absorbed, may also complicate the quantitative absorption of the compound [14]. Thus, for improved oral absorption, it is important to protect the formulation from hepatic metabolism and increase its absorption through gastrointestinal tract. The following sections describe the oral CsA administration approaches tried so far.

Ciclosporin was first marketed as an oil-based oral solution (Sandimmunee®; Novartis), which was based on olive oil, Labrafil® (peglicol-5 oleate; Gattefossé), and alcohol [15]. Upon dilution in the aqueous phase, this formulation forms a crude oil-in-water (o/w) emulsion. For the subsequent formation of mixed micelles promoting the absorption of CsA, the presence of bile and pancreatin is mandatory. Therefore, the Sandimmunee formulation results in comparatively low oral bioavailability, along with a high inter-subject variability [16]. Thus, extensive studies were carried out to improve the oral bioavailability of CsA, which eventually led to a microemulsion formulation with water without the action of bile, Sandimmunee Neoral® [17]. The absorption of the microemulsion is less influenced by bile flow, providing an improved and more consistent bioavailability.

Various dosage forms have also been studied to reduce the toxicity and to increase the absorption of CsA. Particulate polymeric drug delivery systems such as micro- and nanoparticles have been studied extensively. *In vivo* studies with CsA-loaded nanoparticles have demonstrated that polycaprolactone nanoparticles can be used for drug administration to experimental animals with significant increases in oral bioavailability after therapeutic dosing [18]. The pH-sensitive nanoparticles of poly(methacrylic acid and methacrylate) copolymer markedly increase the bioavailability of CsA compared with Neoral, in rats [19]. *In vitro* release experiments have revealed that poly(lactide)-poly(ethylene glycol) particles

Table 1. The main delivery systems developed for ciclosporin A.

Routes	Delivery systems	Advantages	Drawbacks	Ref.
Oral	Emulsion preconcentration for soft gelatin capsule and oral solution (Sandimmune®)	High solubilising capacity	Low oral bioavailability and high inter-subject variability	[15]
	Microemulison preconcentration for soft gelatin capsule and oral solution (Neoral®)	Improved bioavailability	High subject variability	[17]
	Nanoparticles of polycaprolactone	Enhanced bioavailability		[18]
	Nanoparticles of poly(methacrylic acid and methacrylate)	Enhanced bioavailability		[19]
	Nanoparticles of PEG-PLA	Controlled drug delivery		[20,21]
	Positively charged nanoparticles (chitosan HCl or gelatin-A)	Enhanced bioavailability and reduced inter- and intra-individual variability		[22]
	Solid lipid nanoparticle	A low variation in bioavailability and avoiding the plasma peak		[25]
Parenteral	Solution of CsA in Cremphor® EL and ethanol (Cipol®)	Improved solubilisation	Nephrotoxicity	[26,27]
	Liposomes	Improved therapeutic efficacy and reduce nephrotoxicity	Instability	[28]
	Pre-liposomes	Overcome the problems of liposomes during storage		[29,30]
	Cholate-lecithin mixed micelles	Provided alternate for toxic vehicle Cremphor® EL		[32]
	PEO-b-PCL micelles	Sustained drug release and improved thermodynamic stability		[33]
	PLGA nanoparticles	Sustained drug release over long time period		[34]
	Hydroxyapatite microparticles	Biodegradable		[35]
Dermal	CsA in polyethylene glycol-8 glyceryl caprilate	Achieving site-specific immunosuppression		[36]
	Penetration enhancer menthol or SLS (CsA in 40% ethanol)	Enhanced dermal delivery		[38]
	Penetration enhancer monoolein	Enhanced dermal delivery with reduced transdermal permeation		[39]
	Liposomal formulation	Effective in alopecia areata		[40]
	Cubic and hexagonal phases formed by monoolein and water	Enhanced dermal delivery	Induced mild skin irritation	[41]
	Bicontinuous microemulsion	Enhanced transdermal delivery		[42]
	Gelatin-stabilised microemulsion-based organogels	Provided high cutaneous drug deposition *in vitro* and *in vivo*		[43]
	Prodrugs	Enhanced permeability of the drug		[44,45]
	Iontophresis with flexible lecithin vesicles	Enhanced transdermal delivery		[46]
	Low-frequency sonophoresis	Enhanced dermal delivery with reduced transdermal permeation		[48]
	Electroporation	Improved CsA deposition into the skin		[47]
	Microdialysis	Enhanced transdermal delivery		[49]

CsA: Ciclosporin A; HP-α-CD: Hydroxypropyl-α-cyclodextrin; PEG-PLA: Poly(ethylene glycol)-poly(lactide); SLS: Sodium laurete sulfate; PCL: Polycaprolactonel; PEO-b-PCL: Poly(ethylene oxide)-b-poly(ε-caprolactone); PLGA: D,L-lactide/glycolide copolymer; SLN: Solid lipid nanoparticles.

Table 1. The main delivery systems developed for ciclosporin A (continued).

Routes	Delivery systems	Advantages	Drawbacks	Ref.
Pulmonary	Propylene glycol aerosol	Effective preventing graft rejection in the lung transplant patients		[56,57]
	Liposomal aerosol	Enhanced retention in the lung		[58-60]
	CsA/HP-α-CD complex powder	Achieved an acceptable respirable fraction value		[61]
Ocular	Oils	High solubilising CsA capacity	Poor tolerance	[64]
	Cyclodextrins	Enhanced corneal penetration	Repeated administrations	[67]
	Penetration enhancers	Enhanced corneal penetration	Poor tolerance	[68,69]
	Micelles	Enhanced corneal penetration	Poor tolerance and instability	[71]
	Microemulsion	Improvement in dry eye symptoms, FDA approved		[101]
	Emulsion positively charged	Enhanced corneal retention time	Tolerance to be evaluated	[72]
	Prodrugs	Improvement in corneal graft rejection symptoms		[74,75]
	Collagen shields	Enhanced corneal retention time	Patient discomfort	[76]
	PLGA loaded with CsA	Sustained drug release		[77]
	PLGA implants	Provided effective therapeutic level over long time	Surgery for implantation	[81]

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provided a more adequate control of CsA release than conventional PLA micro and nanoparticles [20].

Recently, it has been shown that enhancement of the electrostatic interaction between the mucosal surfaces and drugs have a marked effect on their uptake and overall bioavailability. Thus, El-Shabouri prepared CsA as positively and negatively charged nanopaticles with the aim of improving its bioavailability and reducing its inter- and intra-sample variability [21]. The results revealed that the particle size reduction of CsA to positively charged nanoparticles using cationic polymers such as chitosan HCl or gelatine A improved its absorption rate and bioavailability. However, the oral absorption of CsA nanoparticles, which were prepared by nanoprecipitation with non-biodegradable positively charged polymers (Eudragit® RS and RL; Röhm Pharma GmbH), with or without fatty acid esters (Maisine®; Gattefossé) and polyoxyethylated castor oil (Cremophor®; BASF), were still lower than those observed with the marketed Neoral premicroemulsion [22].

CsA oral delivery systems have also been prepared without particle-forming polymers. CsA nanospheres were prepared by precipitation in an aqueous surfactant solution. However, after oral administration in dogs, the absorption of CsA and the relative bioavailability were poor when compared to the Neoral microemulsion [23]. Liposomes and mixed micelles containing CsA were also studied as carriers after oral administration [24]. However, these formulations were not stable enough and the bioavailability was lower than the presently available marketed dosage forms of CsA. In contrast, solid lipid nanoparticles as a drug carrier for the oral administration of CsA have shown a low variation in the bioavailability of the drug, and simultaneously avoid the plasma peak typical of the first Sandimmune formulation [25]. It might be a promising alternative to the commercial formulations of Sandimmune.

2.2 Intravenous delivery systems

CsA injection is limited to patients who are unable to take the oral preparation, as it has a risk of causing anaphylactic shock and nephrotoxicity due to Cremophor EL, which is used as a solubilising agent used in the commercial intravenous formulation Cipol® (Chong Kun Dang) [26,27].

Several intravenous formulation of CsA, including those using liposomes, microspheres and microemulsion have been investigated to improve the therapeutic efficacy and to remove the need for Cremophor EL. It has been reported that CsA-containing liposomes have shown various depot characteristics and reduced nephrotoxicity [28], but liposomes have several problems associated with their storage, such as phospholipid hydrolysis and decomposition of encapsulated drug [24]. Payne *et al.* introduced drug-loaded proliposomes, a dry free-flowing granular product, which is hydrated immediately before use in order to overcome the abovementioned problems of liposomes [29,30]. Kim *et al.* has also suggested that CsA microspheres and o/w emulsions have sustained release characteristics and have the potential to be used as for intravenous administration of CsA [31].

Cholate–lecithin mixed micelles containing CsA have been shown to exhibit significantly different pharmacokinetic parameters to Sandimmune upon intravenous administration to rabbits, suggesting it might be a suitable alternative to the Cremophor EL present in Sandimmune [32]. Polymeric micelles of methoxy poly(ethylene oxide)-b-poly(ε-caprolactone) have shown high thermodynamic stability, and provided sustained drug release *in vitro* [33].

2.3 Intramuscular and subcutaneous delivery systems

The low and highly variable oral absorption of CsA requires the investigation of controlled release systems that maintain the blood levels of drug within the therapeutic range for a longer time. Sanchez and Alonso have prepared D,L-lactide/glycolide (PLGA) nanopaticles with different sizes and showed that a single subcutaneous dose of microencapsulated CsA provided constant levels of the drug in blood and plasma for extended period of time [34]. In another study, the *in vivo* release of CsA from spherical porous hydroxyapatite microparticles, which was easily injectable, was prolonged to that from the oil preparation by subcutaneous administration [35].

2.4 Dermal and transdermal delivery systems

CsA has been evaluated for numerous potential applications in dermatology. The clinical potential of site-specific immunosuppression with topical CsA has been well recognised. Tran *et al.* were successful in achieving site-specific immunosuppression using a topical formulation of CsA containing polyethylene glycol-8 glyceryl caprilate [36]. However, several attempts to treat psoriasis using topical CsA with or without absorption enhancers have failed to give the expected response, mainly because of the low penetrability of CsA. Although CsA is not a suitable candidate for transdermal delivery, due to 500-Da rule [37], efforts are being made to deliver the drug across the stratum corneum, which is the main barrier for permeation across the skin.

A suspension of 40% ethanol containing 0.5% CsA has been shown to more effectively enhance the topical delivery of CsA after skin pretreatment with 10% menthol or 0.05% SLS [38]. On the other hand, monoolein, a lipidic penetration enhancer, was also found to be effective in improving CsA delivery to the skin. However, the use of a penetration enhancer usually involves pretreatment of the skin, which might cause a change in its nature [39].

In topical and transdermal formulations, the selection of a suitable vehicle is very important, as it can affect both drug release and percutaneous absorption. Many research efforts have been made to improve the delivery of CsA across the skin by various carriers. Verma and Fahr have suggested that 0.5% CsA in a liposomal formulation has promising potential as a topical treatment for alopecia areata in humans [40]. The skin penetration of CsA has been shown to be significantly increased when the drug is incorporated in cubic and hexagonal phases of monoolein and water. However, both phases induced mild skin irritation after 3 days of exposure [41]. Liu *et al.* have suggested that a bincontinuous microemulsion is a promising carrier for transdermal delivery of CsA [42]. In this study, it was also shown that gelatin-stabilised, microemulsion-based organogels (MBGs) show potential as vehicles for the cutaneous drug delivery of CsA. MBGs have been shown to provide a relatively high cutaneous drug deposition *in vitro*. In an *in vivo* study, it was also shown that, by topical applying MBGs, a significant shortening of the time needed to reach steady-state drug penetration (lag time) occurred, as well as an increase in drug disposition into the deeper skin layer, compared with the oral administration. Moreover, the concentrations of CsA in kidney and liver after the dermal application of MBGs were much lower than those of oral administration. It was also implied that the dermal application of the MBGs would be a potential way to achieve the optimal local pharmacological action of CsA on the skin, with minimal systemic side effects [43].

The prodrug approach is an effective means of improving the skin penetration of CsA by chemical derivatisation. The introduction of a polar side chain, either in the form of a positively charged quaternary amine, a negatively charged phosphate or sulfate, or an amphiphilic phosphocholine moiety, has generally been shown to increase the permeability of CsA [44,45]. McGrane *et al.* conjugated a heptamer of arginine to CsA through a pH-sensitive linker to produce R7-CsA. In contrast to unmodified CsA, which fails to the penetrate skin, topically applied R7-CsA was efficiently transported into cells in mouse and human skin [45].

Previous attempts by other investigators to improve the delivery of CsA have also included physical methods. Boinpally *et al.* has suggested that anodal iontophoresis of negatively charged lecithin facilitated the permeation of CsA across human cadaver epidermis *in vitro* [46]. Wang *et al.* have applied an electroporation technique that resulted in the delivery 87 ng of CsA in an 0.87 cm^2 section of mouse dermis; < 0.01 μg in 0.8 ml travelled through the skin to the receiver compartment [47]. Liu *et al.* concluded that the skin accumulation of CsA could be improved by the combination of low-frequency ultrasound and a chemical enhancer, which could help significantly to optimise the targeting of the drug without a concomitant increase of the systemic side effects [48]. In addition, by using intradermal microdialysis in rats, Nakashima *et al.* demonstrated that HPE-101 and glycerine can facilitate the permeation of CsA [49].

2.5 Pulmonary delivery systems

Immunologically mediated lung diseases, such as allergy, hypersensitivity, chronic severe asthma, obliterative bronchiolitis and pulmonary sarcoidosis, have proven to be difficult to treat by conventional oral or intravenous drug therapies, or the therapies are complicated by serious toxic side effects resulting from long-term systemic treatment with CsA [50].

The delivery of medication to the respiratory tract for the localised therapy of respiratory diseases has been practiced for several decades. Recent studies have demonstrated that it is

possible to deliver CsA directly to the lung in an aerosol form [51,52]. Data of canine and rat models indicates that the aerosolised CsA is effective in preventing allograft rejection with a reduced dose compared with intravenous or oral dosage forms [53,54]. A study of aerosolised CsA in lung recipients with refractory chronic rejection has demonstrated that the drug stabilises pulmonary function and can be inhaled without systemic toxicity [55]. Liquid forms of CsA for oral use are not appropriate for atomisation and inhalation. A CsA solution produced using ethanol was the first form for inhalation. However, these forms are irritating due to the ethanol in the preparation. Thus, a new formulation was developed that is based on a solution with propylene glycol. This propylene glycol form has been used for most clinical studies [56,57]. Liposomal preparations have also been developed as alternative forms of the medication, but have not been tested as extensively [58-60].

Besides the formulation of CsA solution for pulmonary delivery, a simple micronised CsA/HP-α-CD complex powder has achieved an acceptable respirable fraction value before and after storage in the dry powder inhalers [61].

2.6 Ocular delivery systems

Over the last decade, extensive reports support the view that the local immunosuppression caused by CsA is effective for the management of corneal graft rejection, autoimmune uveitis and dry eye syndrome [62,63]. Local administration is expected to avoid the various side effects associated with systemic delivery. However, the most popular oil-based vehicles have serious limitations that include the slow partition rate of CsA into the corneal epithelium, and their poor tolerance [64]. Despite these shortcomings, olive oil is still tested in the prevention of corneal graft rejection, and is still the most frequent reference vehicle cited. 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) [65].

A marketed ointment formulation for veterinary use (Optimmune® [Schering-Plough], 0.2% ciclosporin USP ophthalmic ointment) is approved for the treatment of keratocojunctivitis sicca and ocular surface inflammatory diseases in dogs [66]. This formulation has not reached the human field, mainly for its poor acceptability by patients. Besides oil solutions and ointments, many investigations have been made to enhance the solubility of CsA in water by complexation of the drug with cyclodextrins or penetrations enhancers. Cheeks *et al*. have shown on excised rabbit corneas that CsA bound to cyclodextrins results in higher corneal penetration than that of corn oil solution [67]. However, this formulation is limited because of the short residence time on the surface of the eye. CsA in Azone was developed, which resulted in suppression of the severity and incidence of graft rejection. This penetration enhancer has since been shown to induce cytotoxicity on the corneal epithelium [68]. The effect of other penetration enhancers on the transcorneal permeation of CsA has also been investigated [69]. The results have demonstrated that the use of penetration enhancers is a potentially interesting approach, but with the serious limitation of low tolerance of these molecules, due to their modification of corneal properties.

Colloidal carriers are small particles of 10 – 400 nm in diameter, suspended in an aqueous solution. Calvo *et al*. has shown that colloidal particles are specifically taken up by epithelial cells of the cornea by endocytosis [70]. The cornea then acts as a reservoir, releasing the drug to the surrounding tissues. These carriers have provided a means of delivering lipophilic drugs into hydrophilic tissues. Thus, colloidal drug delivery systems were also evaluated for the ocular delivery of CsA, which include micelles, nanoparticle dispersions and liposomes. Although the results of CsA in micelles of a non-ionic surfactant in ocular delivery are promising, a certain number of points remain to be further investigated, such as the ocular tolerance of the surfactant [71]. In addition, micelles are often unstable and their shelf-life must be considered. Ding has developed a castor o/w microemulsion, which received approval from FDA in the form of Restasis® (ciclosporin ophthalmic emulsion, 0.05 wt%; Allergan, Inc.) because of its stability and tolerance [101]. Abdulrazik *et al*. have made a positively charged, emulsion-loaded CsA in order to the prolong residence time on the eye–epithelial corneal cells exhibiting negative charges on their surface; the results are encouraging [72].

Milani *et al*. have applied liposomal technology to the ocular delivery of CsA [73]. However, the large-scale manufacture of sterile liposomes is expensive and technically challenging, which make liposomes hard to apply for CsA ocular delivery.

Nanoparticles, which are able to encapsulate and protect the drug against chemical and enzymatic degradation, improve tolerance, and increase corneal uptake and intraocular half-lifes, have showed promising results over the last 10 years in ophthalmology. However, this approach is not yet completely satisfactory, as precorneal clearance is still too rapid.

Another strategy to enhance the penetration of the lipophilic CsA through ocular tissues is the synthesis of an inactive hydrophilic chemically modified molecule, which can be converted, within the tissues, into the active form after enzymatic transformation. Bourages *et al*. have demonstrated that repeated local administrations of a hydrosoluble CsA prodrug are as efficient as systemic CsA in delaying the corneal graft rejection processes after allogeneic corneal grafting in rats, without detectable CsA systemic levels [74]. In another study, in order to improve the hydrophilicity of CsA, a water-solubilising moiety was grafted on to a free hydroxyl group of the drug. The resulting prodrug was proposed to be a promising candidate in the topical treatment of dry eye disease and corneal graft rejection [75].

Solid systems, including those based on collagen, have also been developed in order to enhance the contact time of the

drug with the extraocular tissue. However, such a device may be difficult for self-administration by patients [76].

The other main routes of administration for ocular therapeutics include the subconjunctival and intraocular pathways. Microspheres and implants have been developed and tested after subconjunctival administration. Harper *et al*. employed microspheres made of 50:50 PLGA copolymer and loaded with CsA [77]. The concentration of the drug in the cornea was maintained for 2 weeks; however, efficacy tests were not performed on animal models of graft corneas. CsA loaded in a liposome suspension has also been manufactured and injected subconjunctivally in rabbits, but liposomes did not offer a significant improvement in terms of the tissue concentrations achieved, compared with free CsA [78].

Degradable PLGA copolymers of composition 85:15 lactide/glycolide was used to manufacture CsA-loaded implants in a disk shape of 5 mm in a diameter and 0.5 mm thickness. The advantage of this approach is that it may provide therapeutic levels of CsA in ~ 15 days in the extraocular area, but its major drawback is its invasiveness [79]. However, the subconjunctival route is very useful when the aqueous humor is the target, and solid forms should allow a better control over release.

Alghadyan *et al*. have demonstrated that liposome-bound CsA can sufficiently maintain therapeutic levels when injected intravitreally [80]. Small PLGA implants have also been placed in the anterior chamber of corneal grafted rabbits, but although the results were promising, further investigations need to determine precisely where CsA is released in the eye [81].

3. Conclusion

Pharmaceutical technology has been shown to successfully improve some of the unfavourable physicochemical properties of CsA. Each of the planned strategies discussed in this article has merit and is promising to the development of a successful delivery system. From all the work that has been reviewed, it appears that biodegradable polymeric nanoparticles are the most promising candidates for oral administration. Liposomal formulations, which are reported to reduce the nephrotoxicity of the drug, are effective alternatives to the toxic solubilising agent Cremphor EL used in the parenteral formulation. Bicontinuous microemulsion, MBGs and some physical or chemical enhancers can be good approaches for dermal delivery, achieving high concentrations in the deeper skin with minimal transdermal permeation, thereby providing site-specific immunosuppression. A simply micronised CsA/HP-α-CD is a very promising formulation for pulmonary delivery because it is capable of efficient lung deposition for a long time without higher systemic absorption. For ocular delivery, chitosan nanoparticles, positively charged emulsions and CsA prodrugs seem to be the most promising candidates. However, none of the described topical systems have really succeeded in achieving therapeutic concentrations for an extended period of time on the corneal surface. Furthermore, sustained therapeutic levels in intraocular tissue can only be achieved by biodegradable and non-biodegradable implants or intravitreal injection. At the present time, despite the need for surgery, the non-biodegradable implant (Vitrasert® [Bausch & Lomb] type) is the most promising device for intraocular delivery, but the ideal system would be biodegradable implants.

4. Expert opinion

The problem with the delivery of CsA is not only a problem of formulation, but also that of a lack of medical knowledge. Several points, such as the mechanism of action, target and therapeutic dose must be more clearly identified and understood. The poor biopharmaceutical properties of CsA require the introduction of novel drug delivery systems, which are at various stages of development. However, at present, only a few formulations of CsA are commercially available and the extensive literature on the delivery of CsA reflects the great medical interest in this challenging drug. Different formulations may have considerably different biological behaviours, and the choice of formulation may affect both short- and long-term clinical outcomes. Presently, there is a lack of clinical comparisons between generic and proprietary formulations, and, thus, bioequivalence can not be assumed. In the future, with advancements in technology, we shall witness an improvement in the management of several disorders of the immune system with transplants and therapy, with the concomitant reduction in the adverse effects associated with certain drugs.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. STAHELIN HF: The history of cyclosporin A (Sandimmune) revisited: another point of view. *Experientia* (1996) **52**:5-13.
2. WENGER RM: Total synthesis of 'cyclosporin A' and 'cyclosporin H', two fungal metabolites isolated from species *Tolypocladium inflatum* Gams. *Helv. Chim. Acta* (1984) **67**:503-515.
3. SCHUMACHER A, NORDHEIM A: Progress towards a molecular understanding of cyclosporin A-mediated immunosuppression. *Clin. Investig.* (1992) **70**:773-779.
4. TAYAR NE: Solvent dependent conformation and hydrogen-bonding capacity of cyclosporine A: evident from partition coefficients and molecular dynamics simulations. *J. Med. Chem.* (1993) **36**:3757-3764.
5. READY A: Experience of cyclosporine. *Transplant. Proc.* (2004) **36**:S135-S138.

• **Comprehensive review of ciclosporin A.**

6. CITTERIO F: Evolution of the therapeutic drug monitoring of cyclosporine. *Transplant. Proc.* (2004) **36**(2 Suppl.):S420-S425.
7. FAULDS D: Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs* (1993) **45**:953-1040.
8. BUSAUSCHINA A: Cyclosporine nephrotoxicity. *Transplant. Proc.* (2004) **36**:2295-2335.

• **Comprehensive review of ciclosporin A nephrotoxicity.**

9. PTACHCINSKI RJ, VENKATARAMANAN R, BURCKART GJ: Clinical pharmacokinetics of cyclosporine. *Clin. Pharmacokinet.* (1986) **11**:107-132.
10. KAHAN BD: Individualization of cyclosporine therapy using pharmacokinetic and pharmacodynamic parameters. *Transplant. Proc.* (1985) **40**:457-456.
11. KELLY PA, WANG H, NAPOLI KL *et al.*: Metabolism of cyclosporine by cytochromes P450 3A9 and 3A4. *Eur. J. Drug Metab.* (1999) **24**:321-328.
12. SAEKI T, UEDA K, TANIGAWARA Y *et al.*: Human *p*-glycoprotein transports cyclosporin A and FK 506. *J. Biol. Chem.* (1993) **268**:6077-6080.
13. LOWN KS, MAYO RR, LEICHTMAN AB *et al.*: Role of intestinal *p*-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin. Pharmacol. Ther.* (1997) **62**:1-13.
14. DREWE J, BEGLINGER C, KISSEL T: The absorption site of cyclosporine in the human gastrointestinal tract. *Br. J. Clin. Pharmacol.* (1992) **33**:39-43.
15. JOHNSTON A, MARSDEN JT, HLA KK *et al.*: The effect of vehicle on oral absorption of cyclosporin. *Br. J. Clin. Pharmacol.* (1986) **21**:P114.
16. FRIMAN S, BÄCKMAN L: A new microemulsion formulation of cyclosporin. Pharmacokinetics and clinical features. *Drug Dispos.* (1996) **30**:181-193.
17. KOVARIK JM, MUELLER EA, VAN BREE JB *et al.*: Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *J. Pharm. Sci.* (1994) **83**:444-446.
18. MOLPECERES J, CHACON M, GUZMAN M *et al.*: A polycaprolactone nanoparticle formulation of cyclosporine improves the prediction of area under the curve using a limited sampling strategy. *Int. J. Pharm.* (1999) **187**:101-113.
19. DAI J, NAGAI T: pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A. *Int. J. Pharm.* (2004) **280**:229-240.
20. GREF R, QUELLEC P, SANCHEZ A *et al.*: Development and characterization of CyA-loaded poly(lactic acid)-poly(ethylene glycol)PEG micro- and nanoparticles. Comparison with conventional PLA particulate carriers. *Eur. J. Pharm. Biopharm.* (2001) **51**:111-118.
21. EL-SHABOURI MH: Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. *Int. J. Pharm.* (2002) **249**:101-108.
22. UBRICH N, SCHMIDT C, BODMEIER R *et al.*: Oral evaluation in rabbits of cyclosporin-loaded Eudraigit RS or RL nanoparticles. *Int. J. Pharm.* (2005) **288**:169-175.
23. FORD J, WOOLFE J, FLORENCE AT: Nanospheres of cyclosporine A: poor oral absorption in dogs. *Int. J. Pharm.* (1999) **183**:3-6.
24. LEE MK, CHOI L, KIM MH *et al.*: Pharmacokinetics and organ distribution of cyclosporin A incorporated in liposomes and mixed micelles. *Int. J. Pharm* (1999) **191**:87-93.
25. MULLER RH, RUNGE S, RAVELLI V *et al.*: Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN) versus drug nanocrystals. *Int. J. Pharm.* (2006) **317**:82-89.
26. CAVANAK T, SUCKER H: Formulation of dosage forms. *Prog. Allergy* (1986) **38**:65-72.
27. BESARAB A, JARRELL BE, HIRSCH S *et al.*: Use of the isolated perfused kidney model to assess the acute pharmacologic effects of cyclosporine and its vehicle, Cremophor EL. *Transplantation* (1987) **44**:195-201.
28. FRESE CE, LIU T, HONG K *et al.*: The increased efficacy and decreased nephrotoxicity of a cyclosporine liposome. *Transplantation* (1994) **57**:928-932.
29. PAYNE NI, BROWNING I, HYNES CA: Characterization of proliposomes. *J. Pharm. Sci.* (1986) **75**:330-333.
30. PAYNE NI, TIMMINS P, AMBROSE CV *et al.*: Proliposomes: a novel solution to an old problem. *J. Pharm. Sci.* (1986) **75**:325-329.
31. KIM SJ, CHOI HK, SUH SP *et al.*: Pharmacokinetic and pharmacodynamic evalution of cyclosporin A O/W-emulsion and microsphere formulations in rabbits. *Eur. J. Pharm. Sci.* (2002) **15**:497-502.
32. GUO J, WU T, PING Q *et al.*: Solubilization and pharmacokinetic behaviors of sodium cholate/lecithin-mixed micelles containing cyclosporine A. *Drug Deliv.* (2005) **12**:35-39.
33. ALIABADI HM, MAHMUD A, SHARIFABADI AD: Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A. *J. Control. Release* (2005) **104**:301-311.
34. SANCHEZ A, ALONSO MJ: Poly (D,L-lactide-co-glycolide) micro and nanospheres as a way to prolong blood/plasma levels of subcutaneously injected cyclosporine A. *Eur. J. Pharm. Biopharm.* (1995) **41**:31-37.
35. MIZUSHIMA Y, IKOMA T, TANAKA J *et al.*: Injectable porous hydroxyapatite microparticles as a new carrier for protein

and lipophilic drugs. *J. Control. Release* (2006) **110**:260-265.

- **Describes an interesting carrier for protein drugs.**

36. TRAN HS, MALLI D, CHRZANOWSKI FA *et al.*: Site-specific immunosuppression using a new formulation of topical cyclosporine A with polyethylene glycol-8 glyceryl caprylate/caprate. *J. Surg. Res.* (1999) **83**:136-140.
37. BOS JD, MEINARDI MM: The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp. Dermatol.* (2000) **9**:165-169.
38. LIU H, LI S, WANG Y *et al.*: Effect of vehicles and enhancers on the topical delivery of cyclosporin A. *Int. J. Pharm.* (2006) **311**:182-186.
39. LOPES LB, COLLETT JH, BENTLEY MV: Topical delivery of cyclosporin A: an *in vitro* study using monoolein as a penetration enhancer. *Eur. J. Pharm. Biopharm.* (2005) **60**:25-30.
40. VERMA DD, FAHR A: Synergistic penetration enhancement effect of ethanol and phosphoilipids on the topical delivery of cyclosporin A. *J. Control. Release* (2004) **97**:55-66.
41. LOPES LB, LOPES JL, OLIVEIRA DC *et al.*: Liquid crystalline phases of monoolein and water for topical delivery of cyclosporin A: characterization and study of *in vitro* and *in vivo* delivery. *Eur. J. Pharm. Biopharm.* (2006) **63**:146-155.

- **Discusses the improved penetration of Ciclosporin A.**

42. LIU H, LI S, WANG Y, HAN F, DONG Y: Bicontinuous water-AOT/Tween85-isopropyl myristate microemulsion: a new vehicle for transdermal delivery of cyclosporin A. *Drug Dev. Ind. Pharm.* (2006) 32:549-557.
43. LIU H, WANG Y, HAN F *et al.*: Gelatin-stabilised microemulsion-based organogels facilitates percutaneous penetration of cyclosporin A *in vitro* and dermal pharmacokinetics *in vivo*. *J. Pharm. Sci.* (2007) (In Press).

- **Complete comparison of percutaneous penetration of Ciclosporin A *in vitro* and *in vivo*.**

44. BILLICH A, VYPLEL H, GRASSBERGER M *et al.*: Novel cyclosporin derivatives featuring enhanced skin penetration despite increased molecular weight. *Bioorg. Med. Chem.* (2005) **13**:3157-3167.
45. MCGRANE PL, WENDER PA, KHAVARI PA: Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. *Nat. Med.* (2000) **6**:1253-1257.
46. BOINPALLY RR, ZHOU, SL, DEVRAJ G *et al.*: Iontophoresis of lecithin vesicles of cyclosporin A. *Int. J. Pharm.* (2004) **274**:185-190.
47. WANG S, KARA M, KRISHNAN TR: Transdermal delivery of cyclosporin A using electroporation. *J. Control. Release* (1998) **50**:61-70.
48. LIU H, LI S, PAN W *et al.*: Investigation into the potential of low-frequency ultrasound facilitated topical delivery of cyclosporin A. *In. J. Pharm.* (2006) **326**:32-38.
49. NAKASHIMA M, ZHAO MF, OHYA H *et al.*: Evaluation of *in-vivo* transdermal absorption of cyclosporin with absoption enhancer using intradermal microdialysis in rats. *J. Pharm. Pharmacol.* (1996) **48**:1143-1146.
50. CORCORAN TE: Inhaled delivery of aerosolized cyclosporine. *Adv. Drug. Deliv. Res.* (2006) **58**:1119-1127.

- **Complete description of inhaled Ciclosporin A.**

51. DOWLING RD, ZENATI M, BURCKART GJ *et al.*: Aerosolized cyclosporine as single-agent immunotherapy in canine lung allografts. *Surgery* (1990) **108**:198-204.
52. MUGGENBURG BA, HOOVER MD, GRIFFITH BP *et al.*: Administration of cyclosporine by inhalation: a feasibility study in beagle dogs. *J. Aerosol Med.* (1990) **3**:1-13.
53. BLOT F, TAVAKOLI R, SELLAM S *et al.*: Nebulized cyclosporine for prevention of acute pulmonary allograft rejection in the rat: pharmacokinetic and histologic study. *J. Heart Lung Transplant.* (1995) **14**:1162-1172.
54. KEENAN RJ, DUNCAN AJ, YOUSEM SA *et al.*: Improved immunosuppression with aerosolized cyclosporine in experimental pulmonary transplantation. *Transplantation* (1992) **53**:20-25.
55. IACONO AT, KEENAN RJ, DUNCAN SR *et al.*: Aerosolized cyclosporine in lung recipients with refractory chronic rejection. *Am. J. Respir. Crit. Care Med.* (1996) **153**:1451-1455.
56. BURKART GJ, SMALDONE GC, ELDON in lung transplant patients. *Pharm. Res.* (2003) **20**:252-256.
57. CORCORAN TE, SMALDONE GC, DAUBER JH *et al.*: Preservation of post-transplant lung function with aerosol cyclosporin. *Eur. Respir. J.* (2004) **23**:378-383.
58. LETSOU GV, SAFI HJ, REARDON MJ *et al.*: Pharmacokinetics of liposomal aerosolized cyclosporine A for pulmonary immunosuppression. *Ann. Thorac. Surg.* (1999) **68**:2044-2048.
59. GILBERT BE, KNIGHT C, ALVAREZ FG *et al.*: Tolerance of volunteers to cyclosporine A-dilauroylphosphatidylcholine liposome aerosol. *Am. J. Respir. Crit. Care Med.* (1997) **156**:1789-1793.
60. KOSHKINA NV, GOLUNSKI E, ROBERTS LE *et al.*: Cyclosporin A aerosol improves the anticancer effect of paclitaxel aerosol in mice. *J. Aerosol Med.* (2004) **17**:7-14.
61. MATILAINEN L, JÄRVINEN K, TOROPAINEN T *et al.*: *In vitro* evaluation of the effect of cyclodextrin complexation on pulmonary deposition of a peptide, cyclosporin A. *Int. J. Pharm.* (2006) **318**:41-48.
62. NUSSENBLATT RB, PALESTINE AG, CHAN CC *et al.*: Doule-masked study of cyclosporine compared to prednisolone in the treatment of endogenous uveitis. *Am. J. Ophthalmol.* (1991) **112**:138-146.
63. MASUDA K, NAKAJIMA A, URAYAMA A *et al.*: Double-masked trial of cyclosporin in Behcet's disease. *Lancet* (1989) **1**:1093-1093.
64. OLIVERO DK, DAVIDSON MG, ENGLISH RV *et al.*: Clinical evaluation of 1% cyclosporine for topical treatment of keratoconjunctivitis sicca in dogs. *J. Am. Vet. Med. Assoc.* (1991) **199**:1039-1042.
65. LALLEMAND F, FELT-BAEYENS O, BESSEGHIR K *et al.*: Cyclosproine A delivery to the eye: a pharmaceutical challenge. *Eur. J. Pharm. Biopharm.* (2003) **56**:307-318.

- **Complete description of ocular Ciclosporin A.**

66. SANSOM J, BARNETT KC, NEUMANN W *et al.*: Treatment of keratoconjunctivitis sicca in dogs with

cyclosporine ophthalmic ointment: a European clinical field trial. *Vet. Rec.* (1995) **137**:504-507.

67. CHEEKS L, KASWAN RL, GREEN K: Influence of vehicle and anterior chamber protein concentration on cyclosporine penetration through the isolated rabbit cornea. *Curr. Eye Res*. (1992) **11**:641-649.
68. NEWTON C, GEBHARDT BM, KAUFMAN HE: Topically applied cyclosporine in azone prolongs corneal allograft survival. *Invest. Ophthalmol. Vis. Sci.* (1988) **29**:208-215.
69. TANG-LIU DD, RICHMAN JB, WEINKAM RJ *et al.*: Effects of four penetration enhancers on corneal permeability of drugs *in vitro*. *J. Pharm. Sci.* (1994) **83**:85-90.
70. CALVO P, ALONSO MJ, VILA-JATO JL *et al.*: Improved ocular bioavailability of indomethacin by novel ocular drug carriers. *J. Pharm. Pharmacol*. (1996) **48**:1147-1152.
71. HICKOK S: Polyoxyethylene stearate. In: Wade A, Weller P (Eds), *Handbook of Pharmaceutical Excipients*. The Pharmaceutical Press, London, UK (1994):379-384.
72. ABDULRAZIK M, TAMILVANAN S, KHOURY K *et al.*: Ocular delivery of cyclosporine A. Effect of submicron emulsion's surface charge on ocular distribution of topical cyclosporin A. *STP Pharma. Sci.* (2001) **11**:427-432.
73. MILANI JK, PLEYER U, DUKES A *et al.*: Prolongation of corneal allograft survival with liposome-encapsulated cyclosporine in the rat eye. *Ophthalmology* (1993) **100**:890-896.
74. BOURGES JL, LALLEMAND F, AGLA E *et al.*: Evaluation of a topical cyclosporine A prodrug on corneal graft rejection in rats. *Mol. Vis.* (2006) **12**:1461-1466.
75. LALLEMAND F, FURRER P, FELT-BAEYENS O *et al.*: A novel water-soluble cyclosporine A prodrug: ocular tolerance and *in vivo* kinetics. *Int. J. Pharm.* (2005) **295**:7-14.
76. REIDY JJ, GEBHARDT BM, KAUFMAN HE: The collagen shield. A new vehicle for delivery of cyclosporine A to the eye. *Cornea* (1990) **9**:196-199.
77. HARPER CA, KHOOBEHI B, PEYMAN GA *et al.*: Bioavailability of microsphere-entrapped cyclosporine A in the cornea and aqueous of rabbits. *Int. Ophthalmol.* (1993) **17**:337-340.
78. ALGHADYAN AA, PEYMAN GA, KHOOBEHI B *et al.*: Liposome-bound cyclosporine: aqueous and vitreous level after subconjunctival injection. *Int. Ophthalmol.* (1988) **12**:101-104.
79. APEL A, OH C, CHIU R *et al.*: A subconjunctivalde gradable implant for cyclosporine delivery in corneal transplant therapy. *Curr. Eye Res.* (1995) **14**:659-667.
80. ALGHADYAN AA, PEYMAN GA, KHOOBEHI B *et al.*: Liposome-bound cyclosporine: retinal toxicity after intravitreal injection. *Int. Ophthalmol.* (1988) **12**:105-107.
81. XIE L, SHI W, WANG Z *et al.*: Prolongation of corneal allograft survival using cyclosporine in polylactide-co-glycolide polymer. *Cornea* (2001) **20**:748-752.

Patent

101. DING S: US5474979 (1995).

- **It has received FDA approval as the first and only therapy for patients with keratoconjunctivitis.**

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