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CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 16 May 2008
Final form accepted: 22 October 2008
Published online: 12 November 2008

Volume 14 (2009) pp 139-152
DOI: 10.2478/s11658-008-0041-6

Mini review

ORAL CYCLOSPORINE A – THE CURRENT PICTURE OF ITS LIPOSOMAL AND OTHER DELIVERY SYSTEMS

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Abstract: The discovery of cyclosporine A was a milestone in organ transplantation and the treatment of autoimmune diseases. However, developing an efficient oral delivery system for this drug is complicated by its poor biopharmaceutical characteristics (low solubility and permeability) and the need to carefully monitor its levels in the blood. Current research is exploring various approaches, including those based on emulsions, microspheres, nanoparticles, and liposomes. Although progress has been made, none of the formulations is flawless. This review is a brief description of the main pharmaceutical systems and devices that have been described for the oral delivery of cyclosporine A in the context of the physicochemical properties of the drug and the character of its interactions with lipid membranes.

Key words: Cyclosporine A, Physicochemical properties, Oral drug delivery, Liposomes, Nanoparticles

INTRODUCTION

Cyclosporine A (CsA) is a major representative of a family of hydrophobic cyclic undecapeptides that exhibit immunosuppressive activity. It is produced by the fungus *Tolypocladium inflatum Gams* and other fungi inperfecti [1], or via

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Abbreviations used: CsA – cyclosporine A; HIV – human immunodeficiency virus; IL – interleukin; NF-AT – nuclear factor of activated T cells; P-gp – P glycoprotein; PCL – polycaprolactone; PEG – polyethylene glycol; PLA – polylactic acid

directed biosynthesis [2] or chemical total synthesis [3]. As early as in 1978, the immunosuppressive action of cyclosporine A was reported to be effective in preventing organ rejections and in the treatment of graft-versus-host diseases. Cyclosporine A is now widely approved for restraining rejection following solid organ transplantations (especially heart, lung and kidney), and preventing and treating graft-versus-host disease after bone marrow transplants. It has also been used in the treatment of numerous autoimmune diseases [4]. However, many additional biological actions of CsA have been reported, including anti-inflammatory, anti-parasitic (anti-malaria), antifungal and antiviral (anti-HIV) action [5].

The drug suppresses T-cell-dependent immune reactions as it inhibits lymphocyte activation by blocking the transcription of cytokine genes for interleukins, in particular IL-4 and IL-2 [6, 7]. Cyclosporine A prevents the translocation of the nuclear transcription factor of activated T cells (NF-AT) from the cytoplasm to the nucleus by reversibly interacting with a cytoplasmic receptor protein, cyclophilin. This complex further binds to calcineurin and inhibits calcium-dependent dephosphorylation of the cytoplasmic component of the transcription factor. Since the activation of gene transcription does not occur, the T cells fail to respond to antigen-mediated stimulation.

Due to the narrow therapeutic range and variable absorption characteristics of CsA, careful blood-level monitoring is essential during therapy [8]. Another drawback to the drug is its broad range of adverse effects, particularly dose-dependent nephrotoxicity characterized by an increase in serum creatinine and urea levels. The mechanisms of nephrotoxicity include the enhanced release of vasoconstrictive factors while vasodilating factor levels are decreased. Although it is generally reversible, CsA-induced nephrotoxicity can progress to chronic injury with irreversible renal damage such as interstitial fibrosis [9]. Moreover, the induction of free radical production by CsA contributes to the nephrotoxicity and to other side effects (e.g. hepatotoxicity, cardiotoxicity) [10, 11]. Below the therapeutic range of CsA, the immune system may not be sufficiently suppressed, leading to organ rejection, but excessive levels of the drug can trigger adverse events. The intra- and inter-individual variations in the bioavailability of most widely used oral formulations of CsA present a major challenge, as some patients receiving the same dose of the drug may experience acute rejection, while others may suffer serious toxicity [12].

THE PHYSICOCHEMICAL PROPERTIES OF CYCLOSPORINE A

CsA is a hydrophobic peptide with a unique structure consisting of 11 amino acid residues, seven of which are N-methylated (Fig. 1). The extensive methylation and hydrophobic character of the amino acid residues together with their four intra-molecular hydrogen bonds, which confer a high rigidity to the cyclic structure, mean the drug has a very low aqueous solubility [13, 14]. It has a molecular weight of 1202.6 Da and its molecular formula is $C_{62}H_{111}N_{11}O_{12}$. CsA is a white or almost white powder, with a crystalline form of white

prismatic needles with a melting point of 148-151ºC [1]. As a dry powder kept in the dark at 2-8ºC, it remains stable for at least 2 years [15], and for over 7 months at 40ºC and 75% relative humidity [16].

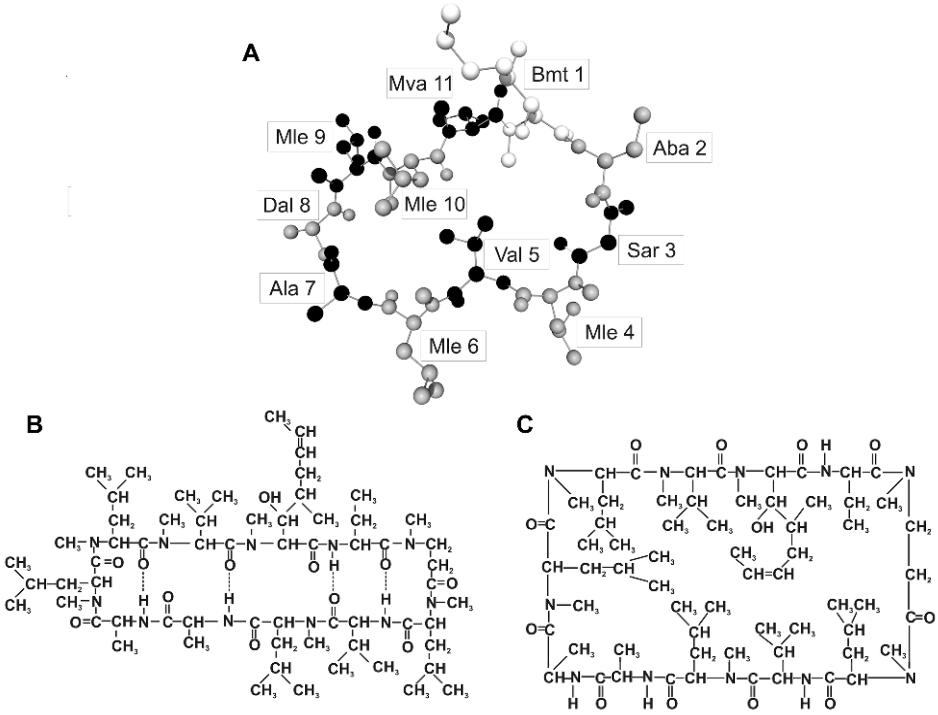

Fig. 1. The chemical structure of cyclosporine A. A – The conformation of CsA in an aqueous environment revealed by the X-ray structure of a CsA-FAB complex (PDB ID: 1IKF) [25]. The abbreviations of the CsA residues are: Bmt, (4R)-4[(E)-2 butenyl]-4,N-dimethyl-L-threonine; Aba, L-a-aminobutyric acid; Sar, sarcosine; Mle, N-methyl leucine; Dal, D-alanine; Mva, N-methyl valine. B – The schematic structure of CsA in crystals [13] and C – in water.

Tab. 1. The solubility of cyclosporine A in various solvents (according to Novartis and [57]).

Solvent	Solubility (mg/g)
Water	0.04
n-Hexane	5.5
Cyclohexane	17
Diisopropyl ether	>20
Acetone	>50
Chloroform; Acetonitrile; Dimethyl sulfoxide; Methanol; Ethyl acetate; Isopropyl alcohol; Ethanol; Polyethylene glycol (200, 300, 400); Propylene glycol; N,N-dimethylacetamide; Glycofurol 75; N-methylpyrrolidone; Sesame oil; Labrafil (WL 2609, M 2125); Labrafac (CC, CM 10); Oleic acid; Tween (20, 80); Solutol HS 15	>100

CsA is generally soluble in organic solvents such as methanol, ethanol, acetone, ether and chloroform (Tab. 1). It is also slightly soluble in saturated hydrocarbons [1] and in water. The aqueous solubility of CsA does not exceed 27.67 μg/ml at 25ºC [17]. It is worth emphasizing that this parameter is temperature dependent in an inversely proportional manner: 106.1 μg/ml at 13ºC and 9.29 μg/ml at 38.5ºC [18]. This phenomenon can be explained by the conformational change of the Dal amino acid residue in position 8. It loses hydration water with increasing temperature, which affects the CsA conformation and consequently its solubility [19, 20]. CsA also lacks ionizable functional groups, so the manipulation of pH does not enhance its solubility. For the same reason, improving solubility by salt formation is not feasible. To overcome these problems, soluble prodrugs have been designed which exhibit up to 25,000 times greater aqueous solubility and could be converted to CsA under physiological conditions [21, 22]. However, these are still in the early stages of development.

As the solubility of the drug is determined by the crystallinity of the solute and its interactions with the solvent, the most efficient way to get a thermodynamically stable increase in solubility is by changing the nature of the solvent. With hydrophobic, non-ionizable drugs such as CsA, the most widely used approaches are cosolvency, micellization and complexation [23]. However, the effectiveness of these three methods on CsA appeared to differ considerably from that on a common model. This indicates conformational changes in the structure of the drug, especially when considered together with the results of partitioning studies of CsA in different solvent systems with different polarities: octanol/water (partition coefficient log P = 2.92) and heptane/water (log P = 1.4) [14]. The dominant conformation in an apolar solvent, the basic features of which are the presence of intramolecular H-bonds stabilizing the structure of a twisted β-sheet and a type II β-turn at amino acid residues 3 and 4, changes dramatically in polar solvents where the molecule exposes its H-bonding groups and loses its secondary structure (Fig. 1) [24-26]. The CsA molecule forms a rigid complex with its receptor, cyclophilin, with half of its residues (Sar3-Dal8) exposed to the solvent and the other residues (Bmt1, Aba2, Mle9-Mva11) buried in the receptor molecule [27]. Despite being less energetically favorable, the conformation in an aqueous environment may make CsA behave as a kind of single-molecular micelle with a higher affinity to water molecules than would be deduced from its structural composition.

LIPID MEMBRANES AND CYCLOSPORINE A

The physicochemical characteristics of CsA are reflected by a rapid exchange of binding sites between the phospholipid membranes with a time constant of about 2.5-5 min. [28, 29]. CsA is not caged in the lipid bilayers, as in pharmacokinetic studies of intravenous CsA liposomal formulations the only critical factors are the absolute lipid amount and the lipophilicity of the drug [30]. The partition coefficient of CsA in a lipid bilayer/water system has been determined via the

liposomal ultrafiltration method as 4000 [31], which was further confirmed in isothermal titration calorimetry experiments (P = 4300 ± 600) [18]. The binding of CsA to lipid membranes follows the classical hydrophobic effect and is accompanied by a positive enthalpy change which must be overcompensated for by a positive entropy change [32]. This is rather unusual, as most peptide-lipid binding reactions involve enthalpy-driven binding. One molecule of the drug could be associated with 19 phospholipid molecules, which appeared to be the upper limit of incorporation in liposomal membranes [18]. A single CsA molecule, the dimensions of which were calculated to be 16.1 x 12.4 Å along the longer axis, occupies an area of 260 $Å^2$ in the lipid monolayer [29]. CsA affects model membranes in a concentration-dependent manner, perturbing lateral organization, in particular in the acyl chain region [18, 33]. Thus, the drug is located in the membrane interior and interacts mostly with the part of the fatty acyl chain proximal to the head group of the phospholipids. Moreover, the drug may cause the separation of a CsA-rich phase in some model bilayers. It was also shown that the undecapeptide preferentially partitions into the boundaries between the fluid and gel domains of the lipids [34]. Other reports investigating the interaction of CsA with model membranes suggest a drug-dependent increase in bilayer fluidity at temperatures below the main phase transition, but an increased order at temperatures above that point [35]. Adding cholesterol to the lipid membrane decreased the binding capacity of the latter for CsA as a consequence of the restriction of acyl chain flexibility, i.e. competition for the same lipophilic binding sites occurs [18, 28]. Thus, the structure of a bilayer influences the nature and topology of CsA inclusion into membranes. This is consistent with earlier observations that the composition of the liposomal carriers of CsA (fatty acid chains and especially the lipid head group) plays a key role in its pharmacokinetic behavior [36].

Due to its lipophilic properties, CsA seems to be an ideal candidate to incorporate into liposomes. Numerous efforts have been made to take advantage of intravenously administered liposomes as carrier systems of CsA to lower its side effects, as liposomes appear to avoid the kidneys [37] but are preferentially cleared by the reticuloendothelial system and tend to accumulate in the spleen, one of the assumed target organs for CsA [30]. Employing liposomes is also part of the attempt to avoid Cremophor EL, the component of the commercial formulation causing renal side effects according to several studies [38]. However, liposome-based delivery systems also have some limitations. As mentioned above, they show a restricted CsA incorporation capacity. Furthermore, cholesterol, a stabilizer of liposomes in the blood, reduces the incorporation levels of the drug. Thus, a compromise must be reached between stability and maximum drug incorporation [28, 39]. Finally, as a result of the significant interlamellar exchange between the carrier and cellular membranes, which makes the controlled release of CsA impossible, the drug is rapidly redistributed while the liposomes are cleared from circulation [28].

SYSTEMS FOR THE ORAL DELIVERY OF CYCLOSPORINE A

Among the various routes of drug delivery, the oral route is the most preferred by the patient and the clinician, so it is no wonder that most of the drugs available today are in the form of tablets or capsules. However, oral administration is a problem in the case of CsA, as it is classified as a Class IV drug under the biopharmaceutics classification system [40], indicating both low solubility [41] and low permeability [42]. One reason is the extensive metabolism of the drug by the cytochrome P-450 3A enzymatic system into 15-30 different metabolites, the toxicity and biological activity of which are significantly less than that of the intact CsA [43]. The process takes place in the liver and to a lesser extent in the gastrointestinal tract and the kidneys, although intestinal metabolism may account for up to 50% when CsA is orally administered [44]. Additionally, CsA is a P-gp substrate, and this ATP-dependent transporter contributes to the efflux of the drug within enterocytes. This phenomenon seems to play a major role in the inter-individual variations in the bioavailability of oral formulations of CsA [45, 46].

Soft gelatin capsules and oral solutions are widely used forms for oral CsA administration. The first registered dosage form (*Sandimmune*®, Novartis, Switzerland) was designed as a crude oil-in-water emulsion preconcentrate [47]. These formulations show a bile-dependent absorption profile and exhibit significant intra- and inter-individual variability. Thus, a new formulation (*Neoral*®, Novartis, Switzerland) was launched on the market over 10 years after *Sandimmune*®. *Neoral*® forms a microemulsion with water without the action of bile [48], providing improved and more consistent bioavailability [49]. A number of generic microemulsion-based formulations of CsA recently became available [50]. However, substituting generic formulations of CsA for *Neoral*® should be considered carefully in light of the fact that standard bioequivalence testing is not adequate to reveal pharmacokinetic variations in individual patients [51]. Therefore, any switch between different formulations should take place only in a controlled setting with adequate pharmacokinetic monitoring.

Since the microemulsion formulations of CsA are far from optimized, various dosage forms have also been considered as potential alternative oral delivery systems (Tab. 2). Although the lipid bilayers of vesicles are unstable in the gastrointestinal tract (which may lead to uncontrolled drug release and its precipitation), liposomes have been found to improve the systemic absorption of CsA after oral administration, and can modify their tissue distribution helping to reduce the nephrotoxicity of the drug [52]. Liposomal CsA was reported to be preferably absorbed by Peyer's patches following oral administration [53]. It was shown that such formulations may exhibit both bioequivalence with microemulsions [54, 55] and diminished inter-individual variations in bioavailability [56]. The problem of the poor shelf-life stability of liposomal CsA formulations can be overcome via the preparation of a dry proliposome powder [55].

Tab. 2. The main oral delivery systems for CsA.

System	Comments	Ref.
Emulsion preconcetration (soft gelatin capsules or oral solution) *Novartis Sandimmune*®	High solubilising efficiency, low bioavailability, high inter-individual variability	[47]
Micromulsion preconcetration (soft gelatin capsules or oral solution) *Novartis Neoral*®	Improved bioavailability, reduced variability in absorption	[48]
Liposomes	Reduced nephrotoxicity and variability in absorption, instability of formulations	[52-56]
Microemulsion based on Solutol HS 15	High solubilising efficiency	[57]
Self-dispersing gels	High degree of cyclosporine solubilization bioavailability close to that of *Neoral*®	[58]
Oil/water emulsions (soybean oil)	Prolonged exposure to the drug	[59, 60]
Solid microspheres of polysaccharides and sodium lauryl sulfate (SLS)	Improved solubilising efficiency, enhanced bioavailability	[61, 62]
Stearic acid nanoparticles	Sustained release effect, bioavailability close to that of to *Neoral*®	[63]
Polysaccharide-based polymeric micelles: polyoxyethylene cetyl ether-grafted dextran and hydroxypropylcellulose	High solubilising efficiency, high stability in gastric and intestinal fluids, no cytotoxicity toward Caco-2 cells	[64]
Particles composed of poly(D,L lactide-co-glycolic acid) (PLGA), poly(D,L-lactic acid) (PLA) and additive fatty acid ester (ethyl myristate; EM)	High entrapment efficiency, factors of size, polymer type and EM influencing the release properties of CyA, biodegradable	[65]
PLGA nanoparticles stabilized by didodecylmethylammonium bromide (DMAB)	High entrapment efficiency, controlled *in vitro* release of CsA, bioavailability higher than that of *Neoral*®, lowered nephrotoxicity	[66]
Poly(lactic acid) and poly(ethylene glycol) (PLA-PEG) micro- and nanoparticles	Improved stability, more adequate control of CyA release	[67]
Polycaprolactone (PCL) nanoparticles	Improved oral bioavailability of CsA and its uptake by lymphocytes *in vitro*, low CsA release	[68, 69]
Eudragit® S100 nanoparticle colloids	More adequate, pH-dependent control of CyA release, bioavailability higher than that of *Neoral*®, improved stability	[70, 71]
Hydroxypropyl methylcellulose phthalate (HPMCP) nanoparticles	pH-dependent control of CyA release, bioavailability close to that of *Neoral*®	[72]
Positively charged nanoparticles (chitosan HCl, gelatin-A or sodium glycocholate (SGC))	Bioavailability higher than that of *Neoral*®, lowered variability	[73]
Polyelectrolyte nanoparticles (poly(ethylenimines) derivatized with cetyl chains and quaternary ammonium groups)	Facilitated absorption within the gastrointestinal tract, high stability, bioavailability close to that of *Neoral*®	[74]
Solid dispersions containing polyoxyethylene (40) stearate	Enhanced drug dissolution, high stability, bioavailability comparable to that of *Neoral*®	[75, 76]
Solid lipid nanoparticles (SLN®)	Controlled drug release, high entrapment efficiency, consistent bioavailability	[20, 77]
Lipospheres	Bioavailability comparable to that of *Neoral*®	[78]
Sugar glasses	Solid dispersions containing fully amorphous CsA, lack of pharmacokinetic data	[79]
Cyclodextrins	Improved bioavailability, reduced variability in absorption	[80, 81]
Prodrugs	High solubility in water, unknown pharmacokinetics	[21, 22]

Micelles composed of modified polysaccharides [64] are one of the promising alternative oral delivery vehicles. Their main advantage involves increasing the apical to basolateral permeability of CsA when compared to free CsA using a Caco-2 cell model. This may be a result of the bioadhesive properties of such micelles. Polymeric micro- and nanoparticles are another extensively studied system. *In vitro* release experiments revealed that poly(lactic acid) and poly(ethylene glycol) (PLA-PEG) particles provided appropriate control of CsA release [67].

Moreover, biodegradable polycaprolactone (PCL) nanoparticles can significantly increase the oral bioavailability of the drug after therapeutic dosing *in vivo* [68], and nanoparticles composed of poly(methacrylic acid and methacrylate) (*Eudragit*®) enhanced the bioavailability of CsA relative to that of *Neoral*® [70]. It was also revealed that the positively charged nanoparticulate formulations of CsA prepared using cationic polymers improved its absorption rate and bioavailability as a result of the electrostatic interactions between the particles and negatively charged mucosal surfaces [73]. A promising alternative to the commercial formulations might be solid lipid nanoparticles. As a drug carrier for the oral administration of CsA, they exhibited a low variation in bioavailability, and simultaneously avoided the plasma peak of the drug typical of *Sandimmune*® [20, 77].

CONCLUSION

The unfavorable physicochemical properties of CsA are associated with its low solubility and permeability, which significantly reduces the bioavailability of the drug and the effectiveness of its oral administration. However, recent improvements in drug delivery technology together with a better understanding of the molecular nature of CsA have allowed the development of a number of promising delivery systems for this peptide. According to some reviews, biodegradable polymeric nanoparticles seem to be the most promising candidates for oral administration. They have not only improved the bioavailability of the drug, but have also increased its overall therapeutic efficacy by targeting the lymphatic system and providing a sustained release of CsA. On the other hand, recent efforts with liposomal CsA formulations show promising results opening up new possibilities of employing them as carriers for the oral delivery of drugs. The extensive literature on the delivery of CsA reflects the great research interest in this field. However, only a few formulations of the drug are commercially available.

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