Reformulating cyclosporine A (CsA): More than just a life cycle management strategy

Melissa Guada a,b,1, Ana Beloqui c,1, M.N.V. Ravi Kumar d, Véronique Préat c, Maria del Carmen Dios-Viéitez a,b, Maria J. Blanco-Prieto a,b,*

Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, C/Tirantacteria 1, E-31008 Pamplona, Spain
Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, Brussels, Belgium
Instituto de Investigación Sanitaria de Navarra, IdiSNA, C/Irunlarrea 3, E-31008 Pamplona, Spain
Department of Pharmaceutical Sciences, Texas A&M Health Science Center, College Station, TX 77845, USA

⁎ Both authors contributed equally to this work.

Abstract
Cyclosporine A (CsA) is a well-known immunosuppressive agent that gained considerable importance in transplant medicine in the late 1970s due to its selective and reversible inhibition of T-lymphocytes. While CsA has been widely used to prevent graft rejection in patients undergoing organ transplant it was also used to treat several systemic and local autoimmune disorders. Currently, the neuro- and cardio-protective effects of CsA (CiCloMulsion®; NeuroSTAT™) are being tested in phase II and III trials respectively and NeuroSTAT® received orphan drug status from US FDA and Europe in 2010. The reformulation strategies focused on developing Cremophor® EL free formulations and address variable bioavailability and toxicity issues of CsA. This review is an attempt to highlight the progress made so far and the room available for further improvements to realize the maximum benefits of CsA.
2. Limitations of CsA
   2.1. Physicochemical attributes
   2.2. Pharmacological attributes
3. Suitable CsA delivery systems: pharmaceutical and clinical considerations
   3.1. Systemic delivery
   3.2. Local delivery
4. Current trends toward the development of novel CsA delivery systems
   4.1. Lipid-based nano/microcarriers
      4.1.1. Ocular route
      4.1.2. Oral route
      4.1.3. Other routes of administration
   4.2. Polymeric-based nano/microcarriers
      4.2.1. Ocular route
      4.2.2. Intravenous route
      4.2.3. Ocular route
   4.3. Other types of carriers
5. Conclusions and future perspectives

References

1. Introduction

Cyclosporine A (CsA) is a well-known immunosuppressive agent that has played a very important role in transplant medicine since the late 1970s. At that time, the fact that it was found to produce selective and reversible inhibition of T-lymphocytes while causing low cytotoxicity won worldwide recognition of CsA as a promising agent in immune therapy. This compound was first isolated from the fungal extract of *Tolypocladium inflatum* in 1973 but its immunosuppressive activity was discovered later by Borel in 1976. After promising outcomes regarding graft survival after renal transplantation, CsA obtained the US FDA’s clinical approval in 1983 for use in prevention of allograft rejection in transplantation. In 1987, the immunosuppressant was registered for the treatment of several autoimmune disorders, and it was in 2003 that the agency approved its use for dry eye disease [1]. Over the years, animal studies and clinical trials have revealed the effectiveness of CsA in other pathologies, such as T-cell large granular lymphocyte leukemia [2], traumatic brain injury (TBI) [3] or ischemic heart disease [4], among others. However, FDA approval has not yet been given for these diseases.

Different CsA formulations are currently available on the market, but there is still a need for improvement. Nowadays, the use of CsA has been limited owing to the related side effects, not only caused by the agent itself but also by the excipients present in the formulations (e.g. high quantities of organic solvents and surfactants). It is also worth mentioning that its unpredictable pharmacokinetics and its narrow therapeutic window are still concerns. In order to overcome these limitations, many promising drug delivery system alternatives based on particulate carriers are now being investigated [5–8]. The scientific efforts devoted to reformulating CsA have been oriented to improve the drug absorption and to modify its tissue distribution. The final goal is to achieve a better pharmacokinetic profile and controlled drug release, thus increasing its therapeutic range, while avoiding the use of Cremophor® as a vehicle, thereby diminishing the number of related side effects.

In this review, innovative CsA delivery systems developed during recent years are summarized, specifically focusing on those consisting on nano- and micro-carriers. The different sections cover (i) the drug background, (ii) the pharmaceutical and clinical aspects that make CsA a challenging drug to formulate, (iii) the critical points to consider for suitable delivery systems depending on the routes of administration, (iv) and current experimental findings and their contribution to the pharmaceutical field.

1.1. Chemical structure and physical properties

CsA (C$_{42}$H$_{61}$N$_{17}$O$_{12}$) occurs as a white powder with a melting point of 148–151 °C, which is barely soluble in water and n-hexane, but highly soluble in other organic solvents and lipids [9]. It has a partition coefficient value (log P) of 2.92 [10]. This lipophilic compound is a neutral cyclic polypeptide consisting of 11 amino acid residues with a molecular weight of 1202.61 Da (Fig. 1). The aminoacids present in the molecule are: (4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine (MeBmt) at position 1, unknown until the isolation of CsA, L-aminobutyric acid (Abu) at position 2, sarcosine (Sar) at position 3, methyl-leucine (MeLeu) at position 4, 6, 9, 10, and 11 is located in the hydrophobic nature of the molecule. The methylamide between residues 9 and 10 is located in the cis configuration and the other remaining fractions are in the trans form. On the other hand, the amide groups at positions 2, 5, 7 and 8 produce four intramolecular hydrogen bonds with the carbonyl groups of residues 5, 2, 11 and 9 respectively, ensuring high rigidity in the structure. Finally, the unsaturated chain at position 1 and the aminoacids at position 2, 3 and 11 are responsible for immunosuppressive activity [11].

1.2. Mechanism of action

The immunosuppressive activity of CsA is attributed to the formation of a complex resulting from the high affinity of the drug with immunophilins, mainly one called cyclophilin A (a cytoplasmic receptor protein of the targeted cells). The CsA-cyclophilin complex formed binds to calcineurin causing the inhibition of its phosphatase activity. Calcineurin is the protein responsible for regulating the nuclear translocation and activation of the nuclear factor of activated T-cells (NFAT) transcription factors. The prevention of the dephosphorylation of NFAT stimulated by the cytosolic calcium hinders their penetration to the core. As a consequence, the transcription of important cytokine genes, including those of IL-2, IL-4, TNF-α and INF-γ, is blocked. Therefore, the proliferation and activation of T-lymphocytes (T-helper and T-cytotoxic cells) are inhibited, the cells do not respond to specific antigen stimulation and thus, the immune system is weakened [12,13].

Furthermore, CsA also binds to cyclophilin D, a protein located in the mitochondria, leading to the blockage of the mitochondrial permeability transition pore (mPTP) and the prevention of mitochondrial mega-
pore formation. This mechanism may be involved in the cardio- and neuro-protective effects attributed to CsA [3,14].

1.3. Pharmacokinetics

CsA is considered a highly variable drug and its efficacy depends greatly on the patient population. Several factors strongly influence CsA disposition through the body and lead to a high intra- and inter-individual variability in the pharmacokinetic parameters. These factors include age, gender, genetics, pathology, diet, dosing time after transplantation, and concomitant administration with other drugs, among others [15].

There are two main routes for CsA administration, intravenous and oral. Although oral administration is preferred, the bioavailability of this lipophilic substance is low and highly variable, ranging from 8 to 60%, with the maximum drug concentration achieved 1 to 8 h after the administration [15–17].

Once in the bloodstream, CsA is widely distributed throughout the body as a result of its lipophilic nature. Its apparent volume of distribution ranges from 2.9 to 4.7 L/kg in humans. From the dose absorbed found in whole blood, CsA is distributed as follows: erythrocytes (58%), plasma (33%), granulocytes (4%) and lymphocytes (5%). In plasma, most of the drug is bound to proteins, mainly to lipoproteins. CsA reaches higher concentrations in lymphoid tissues, such as thymus, spleen, lymph nodes, and bone marrow, rather than in blood. Also, the drug accumulates in lipid-containing tissues, like liver, pancreas, adrenal glands, and adipose tissue, while it barely penetrates into the central nervous system [15].

CsA is largely metabolized in the liver by the oxidation produced by the cytochrome P450 system, specifically by the CYP3A4. Also, the gut wall and the kidney are involved in the drug biotransformation, but to a lesser extent. The cyclic structure of this molecule makes it resistant to metabolism, nevertheless oxidation and demethylation of the side chains lead to the formation of at least 30 metabolites in bile, feces, blood and urine of different species. Some of these metabolites boost the immunosuppressant activity of CsA while others induce toxic effects [17].

Biliary excretion is the main pathway of CsA elimination, which is mostly excreted as metabolites, and only 1% as intact drug. Less important, but also implicated in the drug elimination, is the renal route; approximately 6% of the dose is eliminated in urine. The clearance is approximately 0.35 L/h per kg and the elimination half-life of the drug can vary significantly among patients from mean values of 6.4 h in heart transplantation patients to 20.4 h in patients with hepatic dysfunctions [16].

1.4. Clinical applications

The most important clinical indication of CsA is the prophylaxis of rejection of several transplanted organs, such as kidneys, liver, heart, lung, small bowel, cornea or skin. Moreover, it has been indicated in bone marrow transplantation and graft-versus-host disease. The success of CsA in the transplantation field arises from its selective immunosuppressive effect that allowed it to significantly decrease the rejection rate in the 1980s, and to prolong patient and allograft survival [18]. Due to its successful outcomes in transplantation, the therapeutic application of CsA was extended to the treatment of various autoimmune disorders (Fig. 2). These include severe rheumatoid arthritis, psoriasis, nephrotic syndrome, severe atopic dermatitis, and uveitis, when patients do not respond adequately to conventional therapy [19]. CsA is also used for the treatment of various ocular disorders with evidence of inflammation, like dry eye disease, posterior blepharitis, vernal and atopic keratoconjunctivitis, among others [1]. CsA’s therapeutic activity in treating ulcerative colitis has also been reported [20]. For some physicians, this is the preferred immunosuppressant used as rescue therapy in patients with acute colitis that do not respond to the intravenous steroid treatment, the main reason being that therapeutic levels of CsA can be rapidly reached [21]. Moreover, CsA therapy has been effective in T-cell large granular lymphocyte leukemia and well tolerated regardless of the patient population [2,22]. In the last decade, CsA has attracted special attention as a cardio- and neuro-protective agent. Preliminary data from preclinical studies and early stage clinical trials have demonstrated the beneficial properties of CsA in TBI, stroke and other neuronal conditions [3,4]. Its ability to protect neuronal cells and the mitochondria in the...
cardiac tissue damaged during a heart attack makes CsA a potential candidate for addressing neurological and cardiovascular disorders (Fig. 2). Phase II/III clinical trials are in progress to test CsA’s efficacy in the treatment of these disorders and thus, contribute to the limited existing regimens for these purposes. However, there is some concern about the effective dose-toxicity relation since high doses and chronic administration are needed to evoke the cardio- and neuro-protective effect. Additionally, CsA has exhibited promising results in the treatment of pathologies such as asthma, primary biliary cirrhosis, myasthenia gravis, and insulin-dependent diabetes mellitus, among others [1]. However, more scientific studies are needed for CsA to become part of the established regimens in clinical practice.

1.5. Dosage

The dosing regimen and duration of CsA therapy greatly depends on the patient’s individual condition. The treatment period may last months or years, or may become a lifelong therapy. The therapy is conditioned by the clinical response of the patient and his/her tolerability. For transplantation, the common dose used is 10–15 mg/kg/day of CsA orally within the 12 h prior to the surgery, and is maintained for the first 2 weeks post-transplantation. After this period, this dose is gradually reduced to a maintenance dose of 2–6 mg/kg/day. When the intravenous route is required, the dose is reduced to the third part of the oral dose [23]. Generally, the blood drug concentration is monitored at two hours post dosing (C-2) and the dose is adjusted during the treatment to achieve the desired therapeutic range for an individual patient. The therapeutic CsA C-2 levels can vary from 1000 to 1700 ng/mL during the three first months, depending on the transplanted organ, and followed by a progressive reduction to 600–800 ng/mL [24].

For the treatment of autoimmune diseases, the doses usually employed are lower, starting from 2.5 mg/kg/day of CsA and increasing gradually up to 5 mg/kg/day, if significant clinical enhancement is not observed and the therapy has been well-tolerated. In some cases, discontinuation of the CsA treatment leads to relapse of the pathology [25].

1.6. Adverse effects

Nephrotoxicity is the major concern in patients exposed to CsA therapy. The acute nephrotoxicity is characterized by a reduction of the glomerular filtration rate along with an increase in serum biochemical parameters, such as urea and creatinine. Nevertheless, if the levels of these parameters are carefully monitored in the initial stage of the treatment, the impairment of the renal function can be avoided, since they usually respond to a dose reduction. Inadequate dose adjustment can lead to chronic nephrotoxicity, also related to long-term CsA treatment. In this case, structural damage of the kidney arises and becomes progressive and irreversible, occurring as an interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and glomerulosclerosis [26]. The renal tubular injury is associated with metabolic disorders, including wasting of magnesium, calcium and phosphate as well as distal tubular acidosis, and impaired renal potassium excretion [27]. In turn, the magnesium loss may cause muscle cramps, weakness, paresthesia and sometimes convulsions. Additionally, hypertension is one of the most common pathologies that appear at the initial stage of CsA treatment and is also related to electrolyte imbalance. Presumably, CsA’s mechanism of action is also related to its side effects since the inhibition of the calcineurin-NFAT pathway produced by this molecule is not specific to immune cells. However, other factors have been studied as responsible for renal CsA susceptibility such as the variability in P-glycoprotein and CYP3A4/5 expression or activity, aged kidneys, salt depletion, concomitant medication, and genetic polymorphisms in genes like TGF-β and angiotensin converting enzyme [28].

Other adverse effects that have been reported for CsA therapy include hepatotoxicity, hirsutism, gingival hyperplasia, lymphoproliferative malignancy, etc. [29].

1.7. Commercially available formulations

So far, CsA is available for oral, intravenous and ophthalmic administration (Table 1).
The first CsA formulation on the market was Sandimmune®, supplied as an oral solution or soft gelatin capsules and also as a concentrate solution for intravenous infusion.

Sandimmune® (oral dosage forms) consists of a conventional oil-based formulation containing corn oil, a large amount of ethanol, and inter-esterified corn oil. From this emulsion, CsA absorption is dependent on the presence of bile salts in gastrointestinal environment and its digestion by pancreatic enzymes. As a consequence, the bioavailability of CsA from this formulation has been reported to be low and very variable [30], leading to an erratic relationship between oral dose and total exposure of the compound. Years later Sandimmune Neoral® (hereafter referred as Neoral®) was introduced to the market in order to reach a better pharmacokinetic profile. This is a reformulated product consisting of a preconcentrate microemulsion containing DL-α-tocopherol, ethanol in high proportion, propylene glycol, corn glycerides and Cremophor® RH 40. Unlike the conventional Sandimmune® that forms oil droplets in the micrometric size, the more recent formulation can form homogeneous emulsion droplets of approximately 30 nm immediately after its contact with gastrointestinal fluids, promoting CsA absorption. In this regard, Neoral® has been shown to be less bile-dependent and provide superior and more reproducible bioavailability of CsA, which has been attributed to the micellar solubilization effect and the reduced particle size [31,32]. Despite the better performance in pharmacokinetics for the microemulsion, there is no evidence that Neoral® reduces the risk of side effects arising from Sandimmune® therapy. In addition, achieving sustained constant levels of the drug in blood within the therapeutic window is still a concern, and therefore costly and unpleasant drug monitoring is required [33].

There are other CsA formulations in the market for oral administration, Gengraf®, Deximune® and Panimun Bioral™ as well as several generic formulations; however, they are not bioequivalent [34]. Switching to a different CsA formulation requires supervision of the physicians, and the drug levels must be carefully monitored during the first weeks.

Sandimmune® concentrate for the intravenous route consists of Cremophor® EL and ethanol. It should be diluted in saline solution or 5% glucose before administration. Due to the risk of anaphylactic reactions caused by Cremophor® EL, its use is limited to those cases in which the oral route is not well-tolerated or there are gastrointestinal disorders that threaten drug absorption. Recently, two intravenous CsA formulations have been developed, named CicloMulsion® and NeuroSTAT®, the first one for the treatment of heart reperfusion injury following stenting in patients with myocardial infarction, and the second one for the treatment of severe TBI. Both of them consist of Cremophor® EL free formulations, ready-to-use, which contain physiological fats and phospholipids, characteristics that make them advantageous over the existing marketed formulations. Hence, clinical trials are ongoing in order to obtain the marketing authorization. NeuroSTAT® received orphan drug status from US FDA and Europe in 2010 [35,36].

CsA is also available as an ophthalmic emulsion (Restasis®) containing castor oil, glycerin, polysorbate 80 and carbomer copolymer type A. Furthermore, another two CsA formulations, specifically for veterinary use, are currently commercialized (Table 1). One is Atopica®, an oral formulation indicated in atopic dermatitis; and the other one is Optimmune®, which consists of an ophthalmic ointment based on white petrolatum, used in dogs for the management of keratoconjunctivitis sicca or chronic superficial keratitis.

### 2. Limitations of CsA

Although CsA is available in the market in different dosage forms for different applications and administration routes, its use has been limited owing to certain side effects, which are not only associated with the drug but also with the components used for their preparation. Fig. 3 summarizes some of the pharmaceutical and clinical problems related to CsA, which are explained in more detail in the following sections.

#### 2.1. Physicochemical attributes

Due to its poor biopharmaceutical properties, CsA is a challenging drug to formulate as a suitable delivery system able to ensure not only the efficacy of the drug but also its safety, regardless of the route of administration. Problems associated with CsA include high molecular weight, a rigid structure and a lipophilic nature, which are characteristics that lead to the low solubility of the compound. Consequently, CsA is poorly absorbed across several biological barriers such as the gastrointestinal tract, the stratum corneum and the corneal epithelium, causing an erratic relationship between the administered dose and total exposure, so that the drug concentration achieved in the site of action may be ineffective. Besides, the neutral characteristics of the molecule and the absence of ionizable functional groups make it impossible to obtain a more soluble form of the compound, which is one of the strategies usually employed to achieve improved solubility. Owing to its low solubility and low permeability through the physiological barriers, CsA is classified as Class IV according to the Biopharmaceutics Classification System [23,37]. Nonetheless, this compound has also been classified as Class II according to the same system when surfactants are implicated.

<table>
<thead>
<tr>
<th>Table 1: CsA formulations available on the market.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brand name</strong></td>
</tr>
<tr>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Sandimmune®</td>
</tr>
<tr>
<td>Sandimmune Neoral®</td>
</tr>
<tr>
<td>Gengraf®</td>
</tr>
<tr>
<td>Deximune®</td>
</tr>
<tr>
<td>Panimun Bioral™</td>
</tr>
<tr>
<td>Restasis®</td>
</tr>
<tr>
<td>Atopica®</td>
</tr>
<tr>
<td>Optimmune®</td>
</tr>
</tbody>
</table>

**Fig. 3.** The impact of physicochemical and pharmacological attributes of CsA on its clinical outcomes.
in its formulation [31]. In the search for alternatives to increase CsA solubility, special excipients have been used to formulate the currently marketed formulations. However, they also contribute to the shortcomings of CsA therapy. Ethanol is one of the organic solvents used in both oral and intravenous forms, but it may be harmful for certain patient populations, such as pregnant or breastfeeding women, in patients with hepatic dysfunction or epilepsy, in alcoholic patients or pediatric patients, which restricts its use. Along with this, organic solvents may interact with the shell of the soft gelatin capsules causing the precipitation of some compounds and storage instability [8]. Moreover, one of the solubilizers employed for the microemulsion preparation, Cremophor® EL, might cause gastrointestinal disorders that, as mentioned above, significantly alter drug absorption. Similarly, the concentrator for intravenous infusion contains Cremophor® EL as carrier medium. This solubilizer is known to produce serious side effects, including anaphylactic reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes and peripheral neuropathy [9].

2.2. Pharmacological attributes

The main drawbacks of CsA administration not only involve its limited and variable absorption through the biological barriers, but also its low safety — efficacy correlation. Several factors can explain the deficient CsA performance characterized by the low and unpredictable bioavailability. First, the site of absorption of the compound is limited to a part of the small intestine. Moreover, the P-glycoprotein efflux and the extensive presystemic metabolism in enterocytes and liver can influence the drug levels in the general circulation. The intra- and interindividual variability in bioavailability has been associated with genetic aspects as well as the liver function. Among patients, the polymorphism of the cytochrome P450 system in the liver and enterocytes can differ, so that the drug metabolism and thus, drug concentration would be different between individuals. The production of bile salts and its flow can also affect the drug absorption, this variable being dependent on the patient’s condition. Metabolic state, diarrhea and motility of the gastrointestinal tract are also inherent factors that can alter the permeability of CsA [15]. In transplantation, low levels of CsA can lead to organ rejection, whereas high levels of the drug can result in acute or chronic toxicity. Minimal changes in dose might alter the clinical outcome of the patient. This means that CsA has a narrow therapeutic window and the dosage and the intended indication (Fig. 4). Moreover, the stability of the final product in the different storage conditions is an important aspect to be considered. These delivery systems must ensure efficacy and safety of CsA administration and enable patient comfort and compliance. The following sections focus on the specific considerations required for an optimal CsA performance for the different administration routes.

3. Suitable CsA delivery systems: pharmaceutical and clinical considerations

Several strategies have been investigated to reduce CsA-related side effects. Among these, the co-administration of antioxidants that might induce protective effects against renal injury [38], or the combination with other immunosuppressants in order to minimize CsA dose [19] are the most promising. However, no reliable evidence ensuring patient safety has been demonstrated. Besides, these patients are usually polymedicated so the inclusion of more actives that can interact with the standard treatment is not recommended. In this regard, the best strategy to overcome some of the above-mentioned limitations and enhance the therapeutic efficacy of CsA is to design a suitable CsA delivery system considering some key aspects such as the route of administration, the dosage and the intended indication (Fig. 4). Moreover, the stability of the final product in the different storage conditions is an important aspect to be considered. These delivery systems must ensure efficacy and safety of CsA administration and enable patient comfort and compliance. The following sections focus on the specific considerations required for an optimal CsA performance for the different administration routes.

3.1. Systemic delivery

In transplant and systemic autoimmune disorders CsA delivery by the oral route is preferred. For an optimal CsA systemic delivery, the active should be efficiently and reproducibly absorbed and, once in the bloodstream, target the site of action at therapeutic concentration without compromising safety (Fig. 4). In order to enhance drug oral absorption, it is important to increase the solubility of CsA in the vehicle and keep it dissolved in the gastrointestinal fluid, attempting to prevent drug precipitation in the biological environment. The vehicle has to exhibit high drug loading capacity using a minimum amount of excipients and be as safe as possible. The oral delivery system should be stable in the physiological environment, including pH changes and digestive enzymes, as well as capable of modulating the P-glycoprotein efflux and
avoiding the presystemic metabolism, in order to decrease variability in CsA oral absorption and thus, decrease the risk of acute graft rejection or nephrotoxicity. Rapid release of the drug might be desirable for shortening the time to reach the steady-state concentration and therefore better immunosuppression. Moreover, targeting lymphoid tissue after systemic administration, orally or intravenously, may be advantageous for improving CsA activity on T-lymphocytes. Along with this, sustaining blood levels of CsA within the therapeutic window with a controlled release system can increase dosing intervals and thus enhance patient compliance. For the therapy of neurological disorders, a high concentration of CsA is required to achieve a therapeutic effect. Therefore, in this particular case, it may be advantageous to have a CsA delivery system capable of penetrating the blood brain barrier or/and delivering sustained and localized drug concentrations reaching the desired levels, and also limiting the organ distribution. For the parenteral route, it is important to highlight the use of safe excipients, which avoid the need for Cremophor® EL, which prevents side effects and improves therapeutic efficacy.

3.2. Local delivery

The development of a CsA delivery system for local administration is mainly focused on the skin, cornea and lung, according to the CsA indications. The strategy should consist of achieving the maximal therapeutic effect without compromising the complete immune system of the body. In this way, the adverse effects associated with systemic delivery would be reduced (Fig. 4). For that reason, the delivery system should be able to accumulate high concentrations of CsA in the specific site of action and prevent its distribution to other organs. The vehicle for CsA ophthalmic administration has to be resistant to ocular fluids, increase corneal uptake, be well-tolerated by the corneal epithelium and reduce the precorneal clearance of the drug in order to achieve sustained therapeutic levels in the intraocular tissue for prolonged periods of time. For percutaneous delivery, the vehicle has to facilitate the permeation across the skin, avoiding its irritation and improving drug delivery into the damage tissue. In the development of a pulmonary delivery system, it is expected to target the entire lung tissue providing efficient CsA deposition and retention after inhalation, using an appropriate vehicle for aerosolization able to solubilize CsA but which is harmless to the lungs.

4. Current trends toward the development of novel CsA delivery systems

The present section aims to give an overview of the current state of the art of drug delivery systems for CsA delivery through novel lipid and polymeric drug delivery systems, providing examples of successful outcomes.

4.1. Lipid-based nano/microcarriers

Newly developed lipid-based formulations encapsulating CsA have been mainly exploited via the ocular and oral route (Table 2). The challenge when delivering CsA to the eye is to deliver a CsA therapeutic dose at the targeted ocular tissue with a low toxicity. However, currently available oils to deliver CsA topically to the eye are poorly tolerated and provide a low bioavailability [39]. Here we present examples of different lipid-based drug delivery systems which overcome the aforesaid limitations.

4.1.1. Ocular route

All the reported studies encompassing CsA via the ocular route in the last few years include solid lipid based-formulations [40–44]. Lipid-based nanocarriers have been reported to enhance the bioavailability of ophthalmic formulations [54], particularly in the case of anti-inflammatory drugs [55]. Solid lipid nanoparticles (SLN) are made of biocompatible lipids and present the advantage of avoiding an organic solvent during the preparation method, while presenting a high stability in vivo as they remained solid at body temperature [56], thus representing an alternative also to previous lipid-based formulations (e.g., liposomes). Başaran et al. [41] incorporated CsA (0.1% w/w) into cationic SLN containing Dynasan® or Compritol® as solid lipid and obtained positively charged nanoparticles presenting a mean particle size ~180 nm. The authors chose Dynasan-SLN over Compritol-SLN for in vivo studies as the latter presented a wider distribution size and higher zeta potential. In vivo, Dynasans-SLN were applied topically to sheep and samples from the aqueous and vitreous humor were withdrawn at 2, 16, 24 and 48 h, respectively. The ophthalmic amounts of CsA in vivo in both the aqueous and the vitreous humor (21.30 and 15 ng/mL, respectively) were found to be below the immunosuppressive concentration of CsA, which has been reported to be 0.05–0.30 μg/mL in blood and 0.10 μg/mL in vitreous humor. However, the increased CsA concentrations 48 h upon administration highlights the prolonged CsA released in vivo from SLN compared to previously reported nanoparticles in which CsA concentrations were found to decrease after 8 h [39]. Battaglia et al. [43] evaluated the toxicity of neutral, cationic and anionic SLN ex vivo in rabbit corneas using the bovine corneal opacity and permeability test (BCOP). Regarding SLN toxicity, the authors reported no irritation measured in terms of opacity and permeability. Regarding SLN permeability, higher permeation of fluorescently labeled CsA was reported for SLN compared to CsA emulsion or the drug in suspension. Cationic nanoparticles, obtained by coating SLN with chitosan, exhibited higher permeability values compared to bare nanoparticles (anionic and neutral). Sandri et al. [42] further confirmed these results. Indeed, chitosan-based nanocarriers have been described as a promising platform for oculat therapeutics [57], including CsA administration [58]. Wolska et al. [44] reported that CsA concentration could be increased at least 2% within solid lipid microspheres (SLM) (1–10 μm) compared to the commercial ocular emulsion, while prolonging CsA released for at least 48 h. These findings are in agreement with the data reported by Başaran et al. [41] on CsA release from SLN.

Nanostructured lipid carriers (NLC), a second generation of SLN comprising both liquid and solid lipids, have been also exploited via the ocular route toward CsA delivery. Compared to SLN, these nanoparticles favor increased drug loading due to their unstructured matrix [59]. Shen et al. [40] cross-linked the conjugate of cysteine-polyethylene glycol monostearate (Cys-PEG-SA) into NLC to prepare thiolated NLC (Cys-NLC). Upon topical ocular administration to rabbits, the AUG0–24h and the MRT0–24h of Cys-NLC in aqueous humor, tear and eye tissues were significantly higher compared to those obtained for non-thiolated NLC and an oil solution. The authors attributed these increased concentrations to the ability of thiolated-NLC to prolong the pre-corneal residence time, thus improving CsA distribution in the conjunctiva.

Compared to Restasis® (marketed CsA ophtalmic emulsion), these formulations offer (i) prolonged CsA release that might allow us to lower the daily dose of CsA, (ii) increased CsA encapsulation rates and (iii) good tolerability even at high concentrations. Cationic over neutral or anionic lipid nanoparticles might be more appropriate to obtain increased adhesion into the ocular surface.

4.1.2. Oral route

Most of the studies based on lipid-based formulations aimed at increased CsA bioavailability have been carried out in self-emulsifying drug delivery systems (SEDDS), concretely in self-nanomulsifying drug delivery systems (SNEDDS) [6,47,48]. SNEDDS are clear isotropic fluids. This formulation has been commonly used to improve the solubility of poorly water-soluble drugs and has been demonstrated to prevent the enzymatic and/or chemical hydrolysis of encapsulated drugs [60]. Lei et al. [6] studied...
Table 2  
Lipid-based formulations encapsulating CsA via different routes of administration in preclinical studies.  

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Type of carrier</th>
<th>Ligand grafting/coating</th>
<th>Composition</th>
<th>Outcomes</th>
<th>Ref.</th>
</tr>
</thead>
</table>
| Ocular                 | NLC            | PEG-SA or Cys-PEG-SA    | 70% Preciato®5  
30% Myoglobin®840  
20 wt% CsA  
2 wrr% Tween®80  
2 wrr% PEG-SA  
6% Dinasan®116 or Compritol®888 ATO  
1.01% CsA  
0.1% Octadecylamine  
0.01% Benzalkonium chloride  
4% Tween® 80  
Chitosan 0.2 or 2% (w/w) CsA (10 mg) | • Good ocular tolerance in vivo in New Zealand male rabbits  
• AUCCs.a,24 and MRTcs.a of CsA-PEG-SA NLC in the eye were significantly higher than an oily solution and NLC or PEG-SA-NLC | [40] |
| SLN                    | –              | Coating: 2% Cys-PEG-SA or 2% PEG-SA  
Labrafac® M® 1944 CS,  
Cremophor® EL and Transcutol®P  
8% CsA  
10% PVP K30  
30–70% (w/v) oil (vitamin E, TPGS)  
10–70% (w/v) surfactant (Tween® 20 or 40 or 80 or 80 or Gelucire® or Cremophor® or Cremophor® EL or Cremophor® RH)  
0.30% (w/v) co-surfactant (Labrafac® M® 1944 CS, ethylene glycol, Transcutol, PEG, ethanol, prurol oleique)  
Labrafac® M® 1944 CS (7.6 mg/tablet)  
Transcutol P® (10.1 mg/tablet)  
Cremophor® EL (202 mg/tablet)  
Sucrose (61.6 mg/tablet)  
Lactose monohydrate (61.6 mg/tablet)  
Pregelatinized starch (12.3 mg/tablet)  
PEO N90 (123.3 mg/tablet)  
Pregelatinized starch (12.3 mg/tablet)  
Dinasan®110 or 114 or 116 or 118 or lipo 320  
14% (w/w) Tween® 20  
14% (w/w) Span® 80  
14% (w/w) Cremophor® RH  
7% (w/w) Epikuron 200  
28% ethyl lactate  
14% (w/w) Dinasan® 110 or 114 or 116 or 118 or lipo 320  
5% (w/v) SPC/SDC or SPC/cholesterol CsA (2 mg/mL)  
Compritol®888 ATO  
Poloxamer 188  
Tween® 80  
CsA 1 μg  
1–2.5% HPMC K100M or 1–2.5% Carbopol  
974 P NF  
Lipoid E 80  
Poloxamer 188  
Lipoid MCT | • Prolonged CsA Tmax and MRT, reduced Cmax compared to Neoral® in dogs  
• Bioequivalent with Neoral® in humans  
• Stable at room temperature for over 24 months  
• Improved absorption of CsA in SPC/SDC liposomes compared to SPC/cholesterol liposomes or Neoral® in rats  
• Rapid decrease in ulcer size and increased mucosal repair in an oral ulcer model compared to the untreated group in rabbits  
• Enhanced therapeutic efficacy of ASCs (NP + ASCs) in a myocardial infarction in pigs compared to NP-treated or ASC-treated groups: left ventricular ejection fraction increased, decreased infarct size and neovascularization | [42,43,44,45,46,47,48,49,50,51,52,53] |

wt, weight; w/w, weight/weight; w/v, weight/volume.
differences in CsA absorption between liquid and solid SNEDDS to the particle size (21 nm and 54 nm, respectively) and the dispersing velocity (10 min and 20 min in water, respectively). However, this statement is somehow controversial since the oral bioavailability of CsA containing delivery systems (average particle size of 150 μm × 1000 bigger than Neoral®) has been found to be equivalent to Neoral® in healthy volunteers, thus discarding particle size-bioavailability correlation [32]. Jain et al. [47] evaluated the bioavailability and nephrotoxicity of CsA-TPGS-loaded SNEDDS in vivo in Sprague-Dawley rats and Swiss mice, respectively, and compared to (i) the marketed formulation Bioral™ and (ii, iii and iv) CsA and TPGS alone or in combination, respectively. An increased bioavailability was observed only for CsA-TPGS-loaded SNEDDS compared to Bioral™, which was attributed by the authors to the increased CsA solubilization within TPGS-SNEDDS, the P-glycoprotein inhibition ability of TPGS and the increased encapsulation of CsA within the SNEDDS. Regarding nephrotoxicity, CsA-TPGS-SNEDDS exhibited a significant reduction in nephrotoxicity biochemical markers (creatinine and urea) compared to Bioral™, thus highlighting the safety of CsA-TPGS-SNEDDS over the marketed Bioral™. Zhang et al. [48] formulated CsA-SNEDDS into osmotic pump tablets (SNEOPT) and evaluated CsA bioavailability in dogs. Compared to Neoral®, SNEOPT presented a prolonged T_max and MRT, and significantly reduced C_max. However, similar CsA bioavailability values were obtained.

Avramoff et al. [50] evaluated lipospheres as CsA-loaded lipid-based delivery systems and proved equivalent bioavailability compared to marketed Neoral®. More recently, the authors have improved the formulation, preparing a CsA-loaded liposphere oral pro-dispersion stable at room temperature for over 24 months [49]. Guada et al. [45] evaluated in vitro the immunosuppressive effect of different SLN encapsulating CsA and observed a significant IL-2 secretion decrease in activated Jurkat compared to untreated cells, although an equivalent effect was observed for Neoral®. Likewise, a relative bioavailability of approximately 100% was observed when Precirol LN stabilized with a mixture of L-α-phosphatidylcholine (lec)taurocholic acid sodium salt hydrate (TC) or Pluronic® F127/TC were administered to Balb/c mice using Neoral® as reference formulation. Interestingly, an improved bioavailability was observed for LN containing Tween® 80, attributed to the more resistant properties of Tween® 80 against the gastrointestinal environment. A similar biodistribution profile 24 h-post dosing was obtained for these lipid nanosystems compared to the marketed microemulsion. The authors highlighted the advantages of the novel CsA lipid carriers regarding long-term stability and the safety of the exipients used compared to the commercial formulations [46].

Liposomes can be defined as phospholipid vesicles, encompassing one or more lipid bilayers, consisting of an aqueous core. They can entrap both hydrophilic and hydrophobic compounds [61]. Along with their many advantages (e.g., biocompatibility, large drug payloads, self-assembly capacity), liposomes present certain challenges as far as oral administration is concerned [62]. Probably the most important issue is the instability of liposomes in gastrointestinal fluids, which induces the loss of integrity of the liposomes, thus leading to a reduction in the drug payload dose and resulting in uncertain bioavailability when the drug is administered by the oral route [62]. However, the incorporation of bile salts within the lipid bilayers was found to be able to stabilize the liposomes [63]. Liposomes containing bile salts have been recently exploited as lipid nanocarriers for CsA delivery. Guan et al. [51] evaluated liposomes containing sodium deoxycholate (SDC) bile salt as an oral drug delivery system for CsA. They compared the widely used soybean phosphatidylcholine (SPC)/cholesterol liposomes with SDC/SPC liposomes and observed that both formulations released less than 5% CsA in vitro after 12 h. However, in vivo SPC/SDC liposomes exhibited increased absorption when compared to conventional liposomes or Neoral® in rats (120% versus 98%, respectively, with Neoral® as reference). Although the translation of liposomes to clinical practice has progressed substantially, currently there are no oral CsA liposomal-based therapeutics on the market, or in clinical development [61]. However, the preclinical data about CsA bioavailability obtained upon the oral administration of bile salts-stabilized liposomes seem promising, and their translation into the clinics might be one step closer.

In general terms, CsA-loaded lipid-based nanocarriers exhibited (i) equivalent bioavailability compared to Neoral® (except few exceptions), (ii) decreased toxicity and (iii) long-term stability at room temperature.

4.1.3. Other routes of administration

Recently, a bioadhesive gel formulation containing CsA SLN for the treatment of recurrent aphthous stomatitis has been described [52]. The suitability of the formulation intended for the buccal route was carried out in rabbits in terms of distribution on the buccal mucosa and efficacy in wound healing. After 12 days, the gel containing CsA-loaded SLN showed a statistically significant increased rate of mucosal repair compared to the untreated and the unloaded gel, exhibiting 68% of the formulation retained on the buccal mucosa 6 h after application.

A newly and innovative application of CsA-loaded lipid-based formulations was reported by Yin et al. [53]. In this study, a combination of adipose-derived stem cells (ASCs) with a CsA nanoparticle emulsion (CsA NP) in a swine myocardial infarction model in pigs via the intracoronary route and compared the effect with untreated, CsA NP-treated and ASCs-treated groups. The cardiac function was evaluated 8 weeks later, revealing a significantly increased left ventricular ejection fraction and a significantly decreased infarct size in the ASCs + CsA SLN-treated group compared to CsA NP- and ASCs-treated groups (p < 0.05). Moreover, the ASCs + CsA SLN treatment promoted neovascularization and cardiomyocyte apoptosis (p < 0.05).

4.2. Polymeric-based nano/microcarriers

Polymeric formulations encapsulating CsA have been mainly exploited via the oral, intravenous and ocular route (Table 3). The main matter of discussion regarding especially these routes of administration of CsA is the safety of the formulation. The main aim of these formulations is to increase the absorption of CsA and obtain higher blood concentrations. However, high CsA concentrations in blood lead to nephrotoxicity, among others. In other words, there is a need for a balance in CsA formulations: on the one hand, adequate CsA concentrations for inducing the desired effect, on the other hand, reduced CsA blood levels so that they are innocuous.

Table 2 summarizes the latest polymer-based drug delivery systems tested in vivo. In addition to these examples, several authors have provided interesting data on CsA-loaded polymeric carriers, providing new insights on CsA encapsulation within different types of carriers (e.g. effect of different polymers on CsA encapsulation, different preparation techniques, stability of the formulations, etc). However, these have not been tested in vivo and thus, have not being included within the following table [64–75].

4.2.1. Oral route

As with lipid-based formulations, many efforts have been made in the formulation of CsA within polymeric nano- or microparticles, micelles, microspheres etc. toward an increased bioavailability. Ankola et al. [76] compared conventional PLGA NP (~100 nm) with EL14 (a carbobxylated multi-block copolymer of lactic acid and ethylene glycol) NP (~135 nm) and reported no significant particle size increase in EL14 NP when increasing the drug payload from 10 to 30%, although the entrapment efficiency (EE) tended to decrease. Conversely, PLGA NP exhibited an increased particle size and increased EE. CsA release in vitro was found to be over 90% for both PLGA and EL14 NP, albeit much slower for PLGA NP. In vivo pharmacokinetic studies in rats showed increased C_max, faster T_max and enhanced tissue levels with EL14 NP compared to PLGA NP, and higher bioavailability for both nanoparticles compared to Neoral®. Despite the promising results obtained for EL14, the increased C_max and T_max compromises the safety of the formulation and,
in concrete, might promote the CsA associated nephrotoxicity. Consequently, the authors carried out further studies evaluating the associated nephrotoxicity of CsA-loaded PLGA NP [77]. This study concluded that PLGA NP could reach Neoral® Cmax while decreasing CsA associated nephrotoxicity. Since it reduces CsA-associated nephrotoxicity. These data support previous reports on PLGA’s safety while demonstrating an efficient in vivo mechanism.

Table 3
Polymeric-based formulations encapsulating CsA via different routes of administration in preclinical studies.

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Type of carrier</th>
<th>Ligand grafting/coating</th>
<th>Composition</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral NP</td>
<td>-</td>
<td>CsA</td>
<td>EL14 (50 mg), 0.25% (w/v) DMAB, 2.5 mL ethyl acetate: DCM (1:4), PLGA (50 mg)</td>
<td>• Higher Cmax, faster Tmax and enhanced tissue CsA levels compared to PLGA NP in vivo in rats. • Bioavailability similar to Neoral®</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>CsA (5, 10 or 15 mg)</td>
<td>PLGA (50 mg)</td>
<td>• Bioequivalent Cmax compared to Neoral® in rats. • Significant lower nephrotoxicity</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>CsA (75 mg)</td>
<td>PLGA (500 mg)</td>
<td>• Increased serum drug concentrations despite particle size, exhibiting no toxicity</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>CsA (2 mg/mL)</td>
<td>PVA (1% w/v)</td>
<td>• Significantly increased CsA absorption compared to free drug in suspension (~5-fold) or Neoral® (~2-fold) in rats. • Delayed CsA absorption with Tmax varying from 3.7 to 9 h and significantly lower Cmax compared to Neoral® in rats.</td>
</tr>
<tr>
<td>pH-sensitive NP</td>
<td>-</td>
<td>CsA</td>
<td>GCPQ (15 mg/mL)</td>
<td>• Higher Cmax, AUCo–v and AUCO–∞ (178%) compared to Neoral® in vivo in Beagle dogs. • Facilitated absorption over increased release</td>
</tr>
<tr>
<td>Cubic NP</td>
<td>-</td>
<td>CsA</td>
<td>GMO (500 mg), Poloxamer 407 (40–100 mg)</td>
<td>• Inhibited expression of IL-1β, IL-6 and TNF-α in vitro in LPS-activated macrophages. • Significant colitis amelioration compared to untreated group in a DSS-induced murine colitis model. • Significantly increased oral bioavailability compared to Neoral® (~1.35-fold) in rats. • Elimination half-life of the NP was 21-fold longer compared to a CsA solution and the AUC ~26-fold larger in rabbits. • Improved stabilizing properties due to PEG moieties.</td>
</tr>
<tr>
<td>Microspheres</td>
<td>-</td>
<td>CsA (10 mg)</td>
<td>PLGA (200 mg)</td>
<td>• Selective accumulation in the liver. • Absence of toxicity compared to free CsA treatment. • Decreased immunosuppressive effect compared to free CsA. • Inhibited HCV replication in an HCV mouse model.</td>
</tr>
<tr>
<td>Micelles</td>
<td>mPEG</td>
<td>CsA</td>
<td>Soybean lecithin, mPEG-chitosan Poloxamer</td>
<td>• Targeted immunosuppression to the lymph nodes in mice after intravenous administration of CsA NP-loaded DCs. • Selective accumulation in the liver. • Absence of toxicity compared to free CsA treatment. • Decreased immunosuppressive effect compared to free CsA. • Inhibited HCV replication in an HCV mouse model.</td>
</tr>
<tr>
<td>Intravenous NP</td>
<td>mPEG</td>
<td>CsA</td>
<td>PLA (10 mg)</td>
<td>• Equivalent immunosuppressant effect in mice compared to Sandimmune®</td>
</tr>
<tr>
<td></td>
<td>LTP</td>
<td>CsA (5 mg)</td>
<td>PLGA (50 mg)</td>
<td>• Significant CsA concentration with PLGA + Eudragit® RL (25:75) in rabbit tears compared to Restasis® (AUCo–∞: 972.59 vs 514.24 ng h/g, respectively; Cmax: 366.30 vs 299.02 ng/g, respectively).</td>
</tr>
<tr>
<td>Micelles</td>
<td>Carbopol®</td>
<td>CsA (5 mg)</td>
<td>PEO-b-PCL (30 mg)</td>
<td>• Significant CsA concentration with PLGA + Eudragit® RL (25:75) in rabbit tears compared to Restasis® (AUCo–∞: 972.59 vs 514.24 ng h/g, respectively; Cmax: 366.30 vs 299.02 ng/g, respectively).</td>
</tr>
<tr>
<td>Ocular NP</td>
<td>Carbopol®</td>
<td>CsA (10 mg)</td>
<td>PLA (50 mg) or Eudragit® RL (50 mg) or PLGA + Eudragit® RL (75:25, 50:50, 25:75% (w/w))</td>
<td>• Significant CsA concentration with PLGA + Eudragit® RL (25:75) in rabbit tears compared to Restasis® (AUCo–∞: 972.59 vs 514.24 ng h/g, respectively; Cmax: 366.30 vs 299.02 ng/g, respectively).</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>CsA (10 or 20% of the polymer) Chitosan (2 g)</td>
<td>2% (v/v) acetic acid solution 96% ethanol solution</td>
<td>• CsA estimation in both the aqueous and the vitreous humor 72 h after topical administration in sheep</td>
</tr>
<tr>
<td>Micelles</td>
<td>-</td>
<td>CsA (26.4 mg)</td>
<td>mPEG-hePBLA (120 mg)</td>
<td>• Significant lower edema and increased transparency in a rat cornea transplanted model compared to the untreated group. • Higher CsA cornea levels compared to the systemic treatment</td>
</tr>
</tbody>
</table>

w/w: weight/volume; v/v: volume/volume; wt: weight; w/w: weight/weight.
Cubic NP, made of GMO and poloxamer 407, encapsulating CsA have also been evaluated toward increased CsA bioavailability [80]. The relative AUC0−∞ of CsA in dogs compared to Neoral® was found to be 178%. The authors correlated the enhanced CsA bioavailability with facilitated absorption rather than improved drug release.

Yu et al. [82] evaluated supersaturated micelles made of Soluplus®, a graft amphiphilic polymer. Following an in vivo pharmacokinetic study in rats, the authors reported an increased AUC0−t, T_max and C_max with a relative bioavailability of 134%, compared to Neoral®. However, this was only achieved with one of four supersaturation degrees, in concrete, with 3.53 (drug/Soluplus® ratio, 1/7), and increasing supersaturation degrees led to decreased oral absorption (p < 0.01). These data illustrate a dissolution and solubility-limited oral absorption of CsA.

pH-sensitive NP have been described as promising for oral peptide/protein delivery. Different examples, including CsA loading pH-sensitive NP, are included in the review written by Wang et al. [88]. Dai et al. [8] developed pH-sensitive NP made of medical-grade nanoporous silica (Sylysia 350) and Eudragit®. CsA-loaded NP exhibited a relative bioavailability of 134%, compared to Neoral® after orally administered to rats. However, it was worth noting that the CsA blood concentrations detected within the Neoral®-treated group were found to be beyond the CsA concentrations that have been reported to lead to severe nephrotoxicity [89] and thus, pH-sensitive NP might also represent an alternative toward innocuous CsA oral formulations.

All in all, polymeric-based drug delivery systems encapsulating CsA for oral delivery present increased bioavailability compared to marketed CsA formulations. However, the high accumulation of CsA in different organs and the rapid absorption might compromise the safety of these formulations. The associated CsA nephrotoxicity has only been evaluated in few cases, and mainly for PLGA-based NP. Exhaustive long-term dose-toxicity studies should be carried out prior to the translation of these formulations to clinical practice. Nevertheless, the increased CsA absorption within these formulations is undeniable.

4.2.2. Intravenous route

Probably the most widely exploited strategy to achieve prolonged circulation time of the formulations in the bloodstream is PEGylation, as PEG chains are known to provide “stealth” properties [90]. As an example, by PEGylating NP surface, the NP half-life is prolonged and it is known to decrease their recognition by the reticulo-endothelial system (RES). This approach has also been exploited to deliver CsA following the intravenous route. This is the case of the study reported by Zhang et al. [7]. These authors grafted mPEG to chitosan and then prepared mPEG-chitosan nanoparticles encapsulating CsA and lecithin in their inner core. After being intravenously administered, the PEG-modified chitosan NP exhibited an elimination half-time 21-fold longer than CsA in solution and an AUC −26-fold higher. Additionally, the authors reported that PEG chains (i) provided the NP with stabilizing properties, (ii) hindered the interaction with plasma proteins, (iii) reduced the number of NP taken up by the RES, (iv) prolonged the retention time of the NP and (v) improved the bioavailability of the NP. Jyothi et al. [84] conjugated a liver-targeting peptide (LTP) to PEGylated CsA-encapsulated PLGA NP. The authors used these NP to treat hepatitis C virus (HCV) in a HCV murine model, thus using CsA as antiviral agent. The HCV-NP treated group showed a sustained anti-HCV effect after a short-term treatment (21 days) while minimizing the liver and kidney toxicity compared to free CsA treatment. These are promising data as the applicability of CsA as antiviral agent is hampered by its related nephrotoxicity and hepatotoxicity that have limited its use in clinical practice.

An innovative and smart alternative for targeted immunosuppression was described by Azzi et al. [83]. In order to exploit the ability of CsA in suppressing T-cell mediated-responses, the authors aimed at targeting PLGA containing CsA-PLA to the lymph nodes, which represent the primary site where naïve T cells meet antigen presenting cells inducing them to become alloreactive. Following the presumption that dendritic cells (DCs) would phagocyte the NP and then migrate into the lymph nodes, the researchers coupled dendritic cells (DCs) with CsA-NP. The conjugated NP technique on CsA would protect DCs from cell death. The authors successfully demonstrated CsA-NP internalization by DCs in vitro, exhibiting no apoptosis, in contrast with free CsA-treated DCs. After the injection of coupled DCs into mice footpads, the authors showed efficient trafficking of DC to the lymph nodes. Interestingly, compared to uncoupled DCs, CsA-NP-treated DCs efficiently suppressed the proliferation and activation of CD8 T cells in the lymph nodes.

Hamdy et al. [5] developed PEO-b-PCL micelles encapsulating CsA and evaluated their immunosuppressive effect in vivo and in vitro. In vitro, the inhibitory effect of CsA on the allostimulatory ability of DCs was assessed. However, the effect was comparable to that observed for Sandimmune®. The same effects, and the results, were further confirmed in vivo. Nevertheless, CsA-micelles represent an alternative for the delivery of CsA with the advantages of prolonged drug release and reduced risk of nephrotoxicity.

These results call for further studies focused on targeted immunosuppression using CsA as immunomodulator.

4.2.3. Ocular route

In the case of ocular formulations, a major goal is to maintain the therapeutic effects for an adequate period of time as the liquid forms can be easily removed from the eye. The rapid renewal rate of the lachrymal fluid (1–3 μL/min) and the blinking reflex, restrict the residence time of drugs in the preconreal space (< 1 min) and, as a consequence, the ocular bioavailability of the instilled drugs (< 5%) [91]. In order to increase CsA residence time, Aksungur et al. [85] prepared PLGA NP and PLGA-Eudragit® RL blended NP. Eudragit® RL was added within the formulation as this polymer provides positive charges, which could interact with the mucins present in the mucus layer, thus increasing NP residence time at the surface of the eye. In addition, the researchers also coated PLGA NP with Carbopol® in order to increase also the adhesiveness of the NP. Different ratios of PLGA:Eudragit® were evaluated. When these NP were tested in vivo in rabbits, the drug concentration in the rabbits’ tear film was higher at all time intervals with a PLGA: Eudragit® NP (25:75) followed by Restasis®. Regarding the kinetic parameters calculated to determine CsA elimination from the preconreal area, the AUC0−t was again significantly higher for PLGA: Eudragit® NP (25:75). Interestingly, this parameter was highly dependent on the nature of the polymers used. The authors attributed the efficacy of the nanocarrier to an increased interaction with the eye surface, rendering an enhanced formulation-mucosa contact and prolonged residence time and thus, increased CsA concentrations in the tear film. Following the aforesaid hypothesis, Başaran et al. [86] prepared positively charged chitosan NP using the spray-drying method. CsA was detected in both the vitreous and the aqueous humor even 72 h after topical administration in sheep.

CsA is routinely used in clinical practice to prevent the cornea rejection after a cornea transplantation using a systemic treatment. Di Tommaso et al. [87] evaluated a micelle-based formulation encapsulating CsA in a rat model for the prevention of cornea graft rejection after a keratoplasty procedure. Following a 14-day topical CsA treatment, three parameters were evaluated: (i) cornea transparency, (ii) edema and (iii) neovascularization. Compared to the untreated group, the micelle-treated group presented significant higher cornea transparency and lower edema 7 and 13 days post-surgery. This effect was comparable to that observed for the systemic treatment, without CsA systemic-related side effects.

4.3. Other types of carriers

There are quite a number of examples in the literature describing drug delivery systems other than nanocarriers for CsA delivery, mainly via the ocular route. Wu et al. [92] described a CsA thermosensitive in
sit forming gel. The gel consisted of hyaluronic acid and a temperature-sensitive polymer (PNIPAAm). Compared to commercial eye drops, the gel exhibited no irritation after it was topically administered to rabbits. Moreover, the conjunctival concentrations of CsA after 24 h of topical administration were significantly higher than those of castor oil solution and commercial eye drops. However, the concentrations were found to be below 10 ng/ml, thus indicating a limited absorption, which could avoid the related systemic side effects.

Eperon et al. [93] prepared CsA and triamcinolone-loaded disks and loaded them into an intraocular lens, aiming at inhibiting uveitis after cataract surgery. This drug delivery system exhibited reduced ocular inflammation after more than 3 months post-implantation.

Gupta et al. [94] developed a novel punctual plug consisting of a hydroxyl ethyl methacrylate core loaded with microparticles and surrounded by a silicone shell. These plugs were able to deliver CsA for 3 months at zero-order at a 3 μg/day rate.

Rodriguez-Aller et al. [95] evaluated concentrated eye drops containing a CsA prodrug, solubilized in water. The prodrug solutions were tested in vivo at increasing CsA concentrations (0.05–2% w/v CsA). Each prodrug formulation was compared to conventionally used CsA eye drops at an equivalent concentration. The in vivo results showed that the prodrug formulation led to higher corneal and conjunctival levels than the CsA formulations.

5. Conclusions and future perspectives

Developing novel drug delivery systems for CsA administration remains a challenge. The balance between efficacy and safety in CsA therapy has not been resolved yet and therefore, the costly and unpleasant monitoring for patients is still required. The scientific community has made an enormous effort to improve the available CsA formulations. The major concerns still remain its variable pharmacokinetics and the excipients used in the formulation of this drug. It is obvious that there is increasing interest in this immunosuppressant for use in daily clinical practice: researchers are looking for an ideal vehicle able to give the maximum CsA efficacy after local or systemic delivery while avoiding as far as possible its related side effects.

The literature is rich on publications concerning formulations which successfully encapsulated CsA via different routes of administration, using different types of drug delivery systems and for treating different diseases. The examples included in this manuscript specially highlight nano- and microcarrier-based drug delivery systems as promising alternative formulations to those currently being marketed. The examples of CsA-containing formulations herein described broaden the applicability of CsA. An example is the success of CsA as an antiviral agent in treating hepatitis C.

Although examples of CsA drug delivery systems via percutaneous and pulmonary route have not been extensively described in recent studies, the skin and the lungs represent promising routes of administration for CsA local therapy, as has been reported [96–99].

Neoral®, NeuroSTAT® and CicloMulsion® contain CsA in solution as a lipid emulsion (lipid formulations). Interestingly, more efforts have been made within the last five years in order to foster the advancement in polymeric rather than lipid formulations. The development of techniques for the optimum characterization and visualization of lipid-based formulations (e.g. cryo-electron microscopy) has been probably the reason for the increased interest in these formulations [59]. The number of formulations containing Cremophor® EL has been dramatically decreased and has been replaced mainly by polymers that have exhibited no related side effects in vivo. From a manufacturing point of view, the scale up of a solvent-free formulation is easier than for a formulation containing an organic solvent, and also safer, as it avoids residual solvents that could be harmful in a clinical product. This applies to formulations presenting increased stability (e.g. bile salt-containing liposomes) that ease the handling of formulations that otherwise could not possibly be scaled up.

One of the major hurdles in CsA delivery is its innate toxicity that induces, among other effects, nephrotoxicity and liver toxicity. This has represented a major concern and is currently a matter for investigation. Fortunately, most of the examples described in this manuscript have overcome these limitations, enhancing CsA's safety profile. More importantly, the toxicity evaluation of CsA-containing formulations has become a must and is present in almost all the studies herein described.

The advances in defeating CsA formulation barriers have led to several clinical trials [36,100,101]. Baiza-Durán et al. [100] substituted the ocular CsA oily emulsion with a micellated aqueous solution, benzalkonium chloride free. Shi et al. [101] implanted poly lactide-glycolide-co-caprolactone (PLGCL) CsA drug delivery systems in the anterior chamber of the eye for suppressing the occurrence of rejection after high-risk keratoplasty. Ehinger et al. [36] assessed bioequivalence after the intravenous administration of CicloMulsion®, a Cremophor EL-free emulsion, exhibiting reduced side effects.

Almost all the drug delivery systems included within this manuscript at least provide comparable in vivo CsA concentrations with regard to Neoral®, if not increasing CsA concentrations, while improving its toxicological profile. There are some examples of clinical trials including newly developed CsA formulations and in view of the data herein included, one might hypothesize that the number of clinical trials including new delivery systems will be increased in the near future.

Acknowledgments

This work has been carried out in the framework of the COST Action TD1004. M. Guada is grateful to “Asociación de Amigos de la Universidad de Navarra” for the fellowship grant. A. Beloqui is a post-doctoral researcher from the Belgian Fonds National de la Recherche Scientifique (F.R.S. – FNRS).


