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Cyclosporine A: A Review of Current Oral and Intravenous Delivery Systems

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ABSTRACT As early as 1978, the immunosuppressive effect of cyclosporine A (CsA), a metabolite of the fungus *Tolypocladium inflatum* (Borel, 1989), was reported to be effective in inhibiting organ rejection in patients receiving kidney transplants from mismatched cadaver donors (Calne et al., 1978) and in the treatment of graft-versus-host disease in patients with acute leukemia following bone marrow transplants (Powles et al., 1978). Today, CsA is still indicated to prevent rejection following solid organ transplantations, prevent and treat graft-vs-host disease following bone marrow transplants, and has also been used in the treatment of autoimmune disease such as psoriasis, rheumatoid arthritis, and nephrotic syndrome (Canadian Pharmacists Association, 2006). The effectiveness of CsA is derived from its ability to specifically and reversibly inhibit immunocompetent lymphocytes in the G_0 and G_1 phase of the cell cycle. The T-helper cells are the main target, but suppression of the T-suppressor cells also occurs. The production and release of lymphokines, including interleukin-2 are also inhibited (Novartis, 2005a). CsA can be administered intravenously as well as orally in the form of a solution or a soft gelatin capsule. The following review will focus on the evolution of the emulsion-based oral formulations from the first generation as Sandimmune® to the second generation Neoral®, both products of Novartis Pharmaceutical, as well as on the Sandimmune® commercial intravenous formulation. The potential of alternative delivery systems, including micelles, micro- and nanoparticles, and liposomes, will also be discussed.

PHYSICOCHEMICAL PROPERTIES, MECHANISM OF ACTION AND CLINICAL USES OF CSA

CsA is a cyclic polypeptide consisting of 11 amino acids as shown in Fig. 1 (Novartis, 2005a). CsA is a white or slightly off-white powder with a melting point of 148–151°C, has a molecular weight of 1202.6 and its molecular formula is $C_{62}H_{111}N_{11}O_{12}$ (Sigma, 1996). It is neutral and highly hydrophobic with poor solubility in water but is generally soluble in organic solvents as shown in Table 1. To overcome poor aqueous solubility, the design of a

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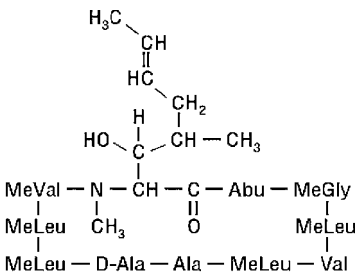

FIGURE 1 **Chemical Structure of Cyclosporine A, (R-[R*,R*-(E)]-Cyclic(L-Alanyl-D-Alanyl-N-Methyl-L-Leucyl-N-Methyl-L-Leucyl-N-Methyl-L-Valyl-3-Hydroxy-N, 4-Dimethyl-L-2-Amino-6-Octanoyl-L-a-Aminobutyryl-N-Methylglycyl-N-Methyl-L-Leucyl-Valyl-N-Methyl-L-Leucyl) (Novartis, 2005a).**

soluble prodrug such as UNIL088, a double ester of CsA, has been investigated. This prodrug exhibited 25,000 times greater aqueous solubility and, through hydrolysis, could be converted back to CsA (Lallemand et al., 2005). CsA partitioning was measured in octanol/water and in heptanol/water systems. The partitioning coefficients log P_{oct} and log P_{hep} were 2.92 and 1.40, respectively (El Tayar et al., 1993).

Biological studies have revealed that CsA inhibits T-cell activation by blocking the transcription of cytokine genes for interleukin-2 (IL-2) and IL-4 (Novartis, 2005a). When a T-cell recognizes a foreign antigen through its T-cell receptor, a cascade of intracellular events occurs including an elevation of calcium levels and subsequent activation of calmodulin. As a result, calmodulin interacts with cyclophilin A which can regulate calcineurin, a superfamily of protein serine/threonine phosphatases. Calcineurin catalyzes the dephosphorylation of NFAT (nuclear-factor of activated T-cells) family members allowing it to translocate into the nucleus and activate gene expression of cytokines such as IL-2 and IL-4 and elicit an immunological response (Novartis, 2005a). Once CsA enters the cell, it binds to cyclophilin located within the cytosol, preventing calcineurin-mediated dephosphorylation. As a result, nuclear translocation of NFAT family members is inhibited as well as gene expression of cytokines deactivating T-cell response (Novartis, 2005a).

CsA is an effective immunosuppressant used to treat patients who have undergone organ transplantation especially heart, lung and kidney transplantation as well as to treat certain autoimmune diseases such as uveitis, psoriasis, and rheumatoid arthritis (Novartis, 2005a). Recently, CsA has been linked as a potential treatment of HIV, hepatitis C and parasites (malaria) although more research in these areas is required (Novartis, 2004, 2005a,b). CsA is a part of a class of calcineurin inhibitors and routinely prescribed in combination with other new immunosuppressive drugs such as mycophenolate mofetil (MMF), sirolimus or azathioprine and prednisone as well as corticosteroids. In addition, a number of studies have suggested that CsA may have neuroprotective effects including extension of life span and reduced neuronal death after weekly intraventricular administration to mice (Scheff & Sullivan, 1999; Karlsson et al., 2004).

Newer analogues of CsA are currently under clinical development including deuterated forms with increased immunosuppressive activity (ISA247; Isotechnica) and nonimmunosuppressive CsA (i.e., NIM811; Novartis and DEBIO-025; DebioPharm). Currently ISA247 and DEBIO-025 are in human clinical trials for immunosuppressive and HIV therapy, respectively.

STABILITY

As a dry powder kept under dark and refrigerated conditions (2–8°C), CsA was stable for at least 2 years (Sigma, 1996) and over 7 months at 40°C, 75% relative humidity (Lechuga-Ballesteros et al., 2003). The oral administration of CsA requires passage through the stomach where the pH is within a range of 1–3. It is, therefore, important to assess CsA's stability under acidic aqueous conditions. When measured at 37°C, its half-lives were found to be 63 hr at pH 1.1 and 79 hr at pH 3.0. Assuming that the gastric emptying half-time is about 50 min, only 1–2% of an ingested dose of CsA would degrade during transit through the stomach into isocyclosporine

TABLE 1 **Solubility Profile of Cyclosporine A in Various Solvents (Novartis, 2004)**

Solvent	Solubility (mg/g)	Solvent	Solubility (mg/g)	Solvent	Solubility (mg/g)
Water	0.04	DMSO	>100	Cyclohexane	17
Acetone	>50	Methanol	>100	n-Hexane	5.5
Chloroform	>100	Diisopropyl ether	>20	Isopropyl alcohol	>100
Acetonitrile	>100	Ethyl Acetate	>100	Ethanol	>100

(Friis & Bundgaard, 1992). Given these results, degradation in the stomach is unlikely to significantly limit bioavailability. Stability has also been measured in blood as well as in fat emulsions. In blood samples, CsA decayed by less than 7.8% in vitro over 5 days (Hows & Smith, 1983; Yonan et al., 2006). When incorporated into fat emulsions of 10–20%, CsA remained stable for up to 48 hr, which demonstrated the compatibility of this drug within emulsions (Jacobson et al., 1993). In the current oral microemulsion formulation Neoral®, which contains 10% CsA in a dissolved state, CsA was reported to be stable for several years (Vonderscher & Meinzer, 1994). Under solid or aqueous acid conditions, in blood, or in fat emulsions, CsA's stability is unlikely to be a factor limiting its bioavailability.

PHARMACOKINETICS

Absorption

The absorption of CsA has been studied in vitro using a Caco-2 cell monolayer as a model of the intestinal barrier. No saturation in the transport of CsA between 1 and 10 μM was seen. In addition, CsA transport exhibited low temperature sensitivity. This suggested that passive diffusion is involved in the uptake of CsA through the intestinal barrier in the apical to basolateral direction. Interestingly, the transport rate in the basolateral to apical direction was greater than that in the opposite direction. This result suggests the involvement of a transporter system operating in the basolateral to apical direction. Vinblastin and daunomycin, known P-glycoprotein substrates, increased the apical-basolateral flux of CsA. This suggested that CsA transport is also influenced by P-glycoprotein on the apical side of enterocytes (Fricker et al., 1996). CsA is absorbed in the ileum and the jejunum.

Distribution

As a result of its lipophilic nature, CsA is widely distributed throughout the body (Atkinson et al., 1982; Lindholm, 1991). In blood, 58% of circulating CsA is bound to red blood cells, 5% to lymphocytes, 4% to granulocytes, and the remaining 33% is in the plasma fraction. Within the plasma, 98% of CsA was bound to proteins. The majority of these proteins were lipoproteins (80–90%) while 5–15% was bound to other proteins such as albumin and globulin. The distribution between lipoproteins was 43–57% to high-density lipoproteins, 25% to low-density lipoproteins and only 2% to very-low-density lipoproteins. Negligible amounts of CsA were bound to chylomicrons (Ptachcinski et al., 1986). CsA concentrations in mononuclear cells were about 1000 times higher than in erythrocytes (Lindholm, 1991). CsA was also extensively distributed within the various tissues of the body. The highest concentrations at about 10-fold greater than in blood were found in fat and the liver. High levels were also measured in leucocyte rich organs such as the thymus, the spleen, the lymph nodes and the bone marrow, as well as in fatty organs including the liver, the pancreas, the kidneys, the adrenal glands, the thyroid, the salivary glands, the lungs, and the skin. The concentration of CsA in muscles was similar to that of the blood. After a single dose, CsA was not detectable in the brain tissue suggesting that this drug does not penetrate the blood-brain-barrier (Lindholm, 1991). However, after chronic use of CsA, low levels were measured in the brain (Atkinson et al., 1982; Lindholm, 1991). Following the cessation of CsA, the drug remained in tissues for over 16 days (Atkinson et al., 1982; Ptachcinski et al., 1986). CsA has also been detected in the amniotic fluid as well as the fetal blood. It has also been found in breast milk (Lindholm, 1991).

Metabolism

CsA was shown to be mostly metabolized by the cytochrome P-450 3A enzyme system in the liver. Metabolism also occurs to a lesser extant in the gastrointestinal tract and the kidney, although intestinal metabolism may account for up to 50% when CsA is administered orally (Hebert, 1997). It is transformed into 15 to 30 different metabolites which have been detected in the bile, feces, blood, and urine (Ptachcinski et al., 1986; Lindholm, 1991; Novartis, 2005a). The toxicity and biological activity of these metabolites were significantly less than that of the parent CsA. The reactions consist of hydroxylation of Cγ-carbon of leucine residues, Cη-carbon hydroxylation, and cyclic ether formation in the side chain of the amino acid 3-hydroxyl-N,4-dimethyl-L2-amino-6-octenoic acid and N-demethylation of N-methyl leucine residues (Novartis, 2005b). Drugs acting as inhibitors or inducers

TABLE 2 **Reported Cytochrome P-450 Inhibitors and Inducers Affecting the Metabolism of CsA (Lindholm, 1991)**

Cytochrome P-450 inhibitors	Cytochrome P-450 inducers
Antibiotics	Antibiotics
Ketoconazole	Rifampicin
Erythromycin	Isoniazide
Josamycin	–
FK-506	–
Pristinamycin	–
Calcium channel blockers	Anticonvulsants
Diltiazem	Phenytoin
Nicardipine	Phenobarbitone
Verapamil	Carbamazepine
Steroid hormones	–
Methylprednisolone	–
Danazol	–
Methyltestosterone	–
Norethisterone	–
Levonorgestrel	–

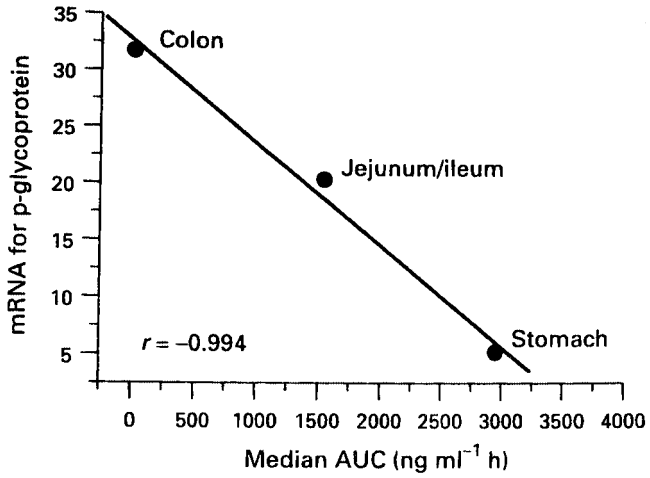

FIGURE 2 **Correlation Between CsA Blood Level AUCs Following the Administration at Distinct Locations within the Gastrointestinal Tract and P-glycoprotein mRNA expression (Fricker et al., 1996).**

of cytochrome P-450 (Table 2) can affect the systemic exposure (AUC) to CsA. Inhibitors such as ketoconazole reduce cytochrome P-450 production, thereby reducing metabolism of CsA, resulting in an increase in the AUC. Inducers will have the opposite effect, resulting in the increased metabolism of CsA and a decrease in the AUC (Lindholm, 1991). Due to the narrow therapeutic index of CsA, co-administration with a drug that interferes with its metabolism may result in clinically significant effects (Pichard-Garcia et al., 2000).

Excretion

The main excretion pathway of CsA is through the biliary system. CsA is mostly excreted as metabolites with less than 1% as unchanged drug. Urinary excretion is only minor. Less than 6% of CsA is excreted through this route, mainly as metabolites (Lindholm, 1991).

INFLUENCE ON DRUG TRANSPORTERS

As described previously, CsA is a P-gp substrate and, within the enterocytes, this ATP-dependent transporter (ATP binding cassette) contributes to the efflux of CsA. It was demonstrated that the median of the AUC of CsA is highly dependent on the location where the dose is administered in the gastrointestinal tract (Fig. 2). CsA administered in the stomach resulted in greater AUC than in the jejunum/ileum, which were greater than in the colon where absorption was poor. It was shown that the extent of absorption of CsA correlated with the concentration of P-gp mRNA. This suggests that absorption of CsA in the lower intestinal tract could be reduced by the increase in P-gp expression (Fricker et al., 1996). Of course, efflux caused by P-gp may not be the only factor influencing absorption as other factors such as metabolism within the enterocytes can also affect the permeability of this drug at these various locations.

The interaction of CsA with other ATP-dependant transporters has been reported within the hepatocyte canalicular membrane including the bile salt export carrier (BSEC), involved in bile salt secretion, the leukotriene export carrier (LTEC), involved in the transport of cysteinyl with glutathione, glucuronide or sulfate moieties, in addition to P-gp. Other transporters are not typically inhibited by substrates of P-gp (Bohme et al., 1993). However, in addition to inhibiting P-gp through competitive binding (Chen et al., 1997), CsA also inhibited the BSEC and LTEC transporters. These results therefore suggest that the administration of CsA could cause cholestasis, which is indicated by a rise in serum bile salts and bilirubin levels because of BSEC. Furthermore, the inhibition of P-gp by CsA, could be used in order to reduce multidrug resistance and delay the elimination of active drugs by the liver (Bohme et al., 1993). The inhibition of P-gp at the apical membrane of enterocytes has also been shown to increase the uptake of bepostatin, an antiallergic agent (Ohashi et al., 2006).

DRUG DELIVERY SYSTEMS

The poor aqueous solubility of CsA had to be taken into account during the formulation of a suitable drug delivery system. Emulsion-based systems were therefore selected and have evolved from a crude oil-in-water emulsion to a microemulsion formulation.

Oral Delivery

Sandimmune® (First Generation)

The first generation oral delivery system, Sandimmune®, was developed in the 1980s as a crude oil-in-water emulsion. It consisted of CsA, alcohol (dehydrated), corn oil and Labrafil M 2125 CS (polyoxyethylated glycolysed glycerides) as well as gelatin and glycerol for the capsule shell, and red iron oxide and titanium dioxide as coloring agents (Novartis, 2005b). CsA was distributed in the oil droplets which had an effective diameter of 3.73 μm (Andrysek, 2003). In order for the drug to be released for absorption, the oil droplets had to be digested. This entailed an emulsification by bile salts thereby allowing their digestion by pancreatic enzymes. The resulting mixture was composed of precipitates of calcium soaps, an aqueous mixed micellar phase in which CsA would be solubilized by a mixture of monoglycerides and bile salts, and the balance of CsA would be dissolved in an oily phase of undigested tri- and diglycerides. This first generation formulation suffered from the delayed release of CsA as a result of this digestion step. Furthermore, because of the bile salts requirement, the physiological state of a patient's gastrointestinal tract, also greatly influenced CsAs release (Vonderscher & Meinzer, 1994). Bioavailability of the Sandimmune® emulsion ranges from 2 to 89% with a mean of 30% (Lindholm, 1991).

Neoral® (Second Generation)

To address the short falls of the first generation of emulsion-based CsA delivery systems, Neoral®, a microemulsion, was developed in 1994. The main objectives were to achieve a rapid release of CsA within the gastrointestinal tract, thereby taking advantage of the full length of the limited absorption window within the small intestine. CsA had to remain in solution when diluted in the aqueous gastrointestinal fluids and avoid precipitation. This formulation also had to allow the incorporation of high concentrations of CsA in a dissolved and stable state using a minimal amount of excipients. The physiological state of the gastrointestinal tract should not significantly influence bioavailability. Finally, the preparation had to be available as either a drinking solution or a capsule thereby ensuring adequate patient compliance. These objectives were achieved through the use of a formulation composed of a lipophilic solvent, hydrophilic solvent and a surfactant that spontaneously form a microemulsion in water (Vonderscher & Meinzer, 1994). This microemulsion preconcentrate will therefore produce a microemulsion when in contact with the gastrointestinal fluids (Noble & Markham, 1995). The formulation includes CsA, DL-α-tocopherol, ethanol, hydrogenated castor oil, maize oil and propylene glycol. It can be administered either as a solution or a capsule with a shell of gelatin, glycerol and propylene glycol, and with the addition of coloring agents such as aluminum chloride, carminic acid, iron oxide black, hydroxypropyl methylcellulose, sodium hydroxide and titanium oxide (Novartis, 2004). The effective diameter of the dispersed particles of the Neoral® microemulsion was measured at 0.03 μm (Andrysek, 2003), about 124-fold smaller than its predecessor Sandimmune®. This microemulsion formulation exhibited greater bioavailability and an improved linear relationship between dose and the area under the curve (AUC) over a wider range of doses compared to the Sandimmune® formulation as shown in Fig. 3

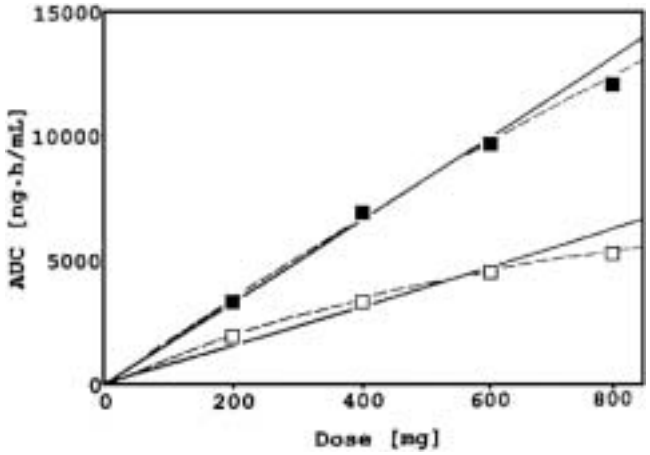

FIGURE 3 **Relationship Between Bioavailability (AUC) and Dose of CsA Following a Single Administration of the Sandimmune® (Open Squares) and Neoral® (Full Squares) Formulations in Healthy Volunteers (Linear: Straight Line and Hyperbolic Function: Dotted Line Regressed through the Origin) (Mueller et al., 1994b).**

(Mueller et al., 1994b). With the Neoral® microemulsion formulation, bioavailability increased by 15–239% compared to Sandimmune® (Mueller et al., 1994a,b; Novartis, 2005a). The time to peak blood concentration (t_{max}) is reached within 1.5 to 2.0 hr following oral administration (Novartis, 2005b). Neoral® therefore offers a more predictable and extensive drug absorption. This may improve the ability of clinicians to predict total drug exposure and possibly reduce the need for regular blood monitoring (Noble & Markham, 1995). The Neoral® microemulsion was found to be as safe as the previous emulsion formulation in renal transplant recipients (Frei et al., 1994; Noble & Markham, 1995).

Micelles and Nanoparticles

The use of micelle-based systems has been investigated as an alternative oral delivery vehicle. Modified polysaccharides have shown some potential. These include dextran-grafted-polyoxyethylene (10) cetyl ether (D-C16) and hydroxypropylcellulose-grafted-polyoxyethylene (10) cetyl ether (HPC-C16). Both of these polysaccharides should be biocompatible as no toxicity was detected when tested using a Caco-2 cell model. D-C16 and HPC-C16 form micelles of 14 nm and 55 nm in size, and are able to load 8.5% and 5.5% CsA w/w, respectively. Their main advantage may be their ability to increase the apical to basolateral permeability of CsA by 1.5-fold (D-C16) to 3.0-fold (HPC-C16) when compared to free CsA. The enhanced performance of HPC-C16 could be a result of its bioadhesive properties (Francis et al, 2005).

Nanoparticles have also been considered as a potential alternative oral delivery system. The use of polycaprolactone (PC) (Molpeceres et al., 2000; Varela et al., 2001) and stearic acid (SA) (Zhang et al., 2000) have been investigated. These nanoparticles have a diameter of 105–115 nm (PC) and 316 nm (SA), can load up to 13% CsA w/w (PC) or 25 μg/mL (SA) and are biodegradable (Molpeceres et al., 2000; Zhang et al., 2000; Varela et al., 2001). When PC nanoparticles were tested in a rat model, the concentration of CsA in the blood, liver, spleen, kidney and urine increased by up to 2-fold compared to Sandimmune®. However, their effect on nephrotoxicity has yet to be fully measured (Varela et al., 2001). Compared to Neoral® the SA nanospheres had a delayed t_{max} of 4.5 hr vs. 1.7 hr and a relative bioavailability of nearly 80% (Zhang et al., 2000).

Intravenous Delivery

Sandimmune® I.V

CsA for injection is available under the Sandimmune® brand. Its formulation consists of CsA mixed with Cremophor® EL (polyoxyethylated castor oil) and alcohol. The concentrate must be diluted with 0.9% sodium chloride or 5% dextrose solution for injection prior to intravenous administration (Novartis, 2005b). Cremophor® EL (CrEL) is a formulation vehicle suitable for hydrophobic drugs which are poorly soluble in aqueous solution. Unfortunately, CrEL is not an inert vehicle. It is known to modulate the activity of P-gp in vitro (Gelderblom et al., 2001). Using a Caco-2 cell model, it was shown that the efflux of CsA (basolateral to apical movement) was inhibited as a function of the CrEL concentration while little to no effect was measured with regards to influx (Seeballuck et al., 2003). Therefore, as a P-gp inhibitor, CrEL could potentially affect the biodistribution of CsA. However, P-gp modulation in vivo by CrEL has not been observed. This lack of efficacy in vivo may be the result of extremely low volumes of CrEL distribution resulting in much low concentrations compared to in vitro conditions (Gelderblom et al., 2001). However, the use of CrEL has several disadvantages including the potential for severe anaphylactoid hypersensitivity reactions, hyperlipidaemia, abnormal lipoprotein patterns, aggregation of erythrocytes as well as peripheral neuropathy (Gelderblom et al., 2001). Alternatives to the CrEL formulation have therefore been investigated. Recently a novel emulsion based CsA formulation under development for I.V. use is NeuroSTAT (NeuroPharma) which is based on Intralipid, the intravenous lipid calorie nutritional supplement.

Liposomes

It is desirable to design delivery vehicles which, when administered systemically, are able to target the site of the allograft, thereby delivering high concentration of CsA locally. This approach could minimize complications caused by systemic immunosuppression thereby reducing mortality and morbidity. The use of liposomes for intravenous CsA delivery has therefore been considered. Liposomes, a phospholipid vesicle, are preferential cleared by the reticuloendothelial system, of which the liver is an important component (Freise et al., 1994). They also preferentially accumulate at sites of infections

and inflammation, and tumor sites. In the absence of disease they do not tend to accumulate in organs such as the heart or kidney (Ouyang et al., 1995). These features may provide selective targeting to tissue rejection sites (Ouyang et al., 1995) or the liver (Freise et al., 1994). CsA has been incorporated in liposomes made of phosphatidylserine/phosphatidylcholine, cholesterol sulfate, and lysophosphatidylserine (3:4:2 ratio) (450 nm) (Freise et al., 1994), or 1-palmitoyl-2-oleoyl-sn-glycero-3-choline (100 nm) with a CsA incorporation of up to 3.6 mol% (Ouyang et al., 1995). Rats treated with a liposomal formulation showed an increased CsA clearance rate, a 2-fold increase in liver tissue to blood concentration as well as increases in spleen and kidney compared to the commercial I.V. CsA formulation. Also, serum creatinine levels return to baseline levels more quickly with the liposomal CsA. The average survival rate of rats following liver allografts when CsA levels were reduced from 2.5 to 1.75 mg/kg/day increased from 51.8 days with commercial CsA compared to 92.6 days with the liposome formulation. The use of liposomes could, therefore, result in early clearance, increase hepatic uptake, and decreased nephrotoxicity in patients receiving liver transplants (Freise et al., 1994). Targeting of the liver was further increased using liposomes modified with bioadhesive polymers. Carbopol 941 modified liposomes, to which CsA was incorporated, increased CsA liver concentration by 2-fold compared to untreated liposomes (Yoshikawa et al., 1997).

Liposome-based delivery systems have several limitations. First, liposomes have a limited CsA incorporation capacity. The hydrophobic CsA incorporates within the lipid bilayer as opposed to being encapsulated in the hydrophilic core. Furthermore, cholesterol content reduces CsA incorporation levels. However, cholesterol plays a role in the stability of liposomes in blood or plasma. A compromise must therefore be reached between stability and maximum drug incorporation (Ouyang et al., 1995; Alangary et al., 1995). Finally, significant interlamelar exchange occurs resulting in a rapid exchange between the carrier and cellular membranes (Ouyang et al., 1995). Controlled CsA release is therefore not possible. As a result, while the liposome carrier is slowly cleared from circulation (>50% remaining after 4 hr), CsA is rapidly redistributed. With the exception of the liver where CsA concentrations were higher, pharmacokinetics were similar for both the liposome and the CrEL formulations (Choice et al., 1995).

Micelles and Nanoparticles

CsA has been successfully incorporated into block copolymer micelles of methoxy (polyethylene oxide)-b-poly(ε-caprolactone) with a 5000–13000 ratio. Micelles of 79 nm were formed with a CsA loading of 13% w/w. This formulation solubilized up to 1.3 mg CsA/mL, which was within the injectable range of 0.5–2.5 mg/mL typically used with CrEL commercial preparation. The main advantage of this micelle system is the ability to obtain a controlled CsA release rate. Only 5.8% of the dose was released within 12 hr compared to 77% for the CrEL formulation (Aliabadi et al., 2005). Mixed micelles systems of dimyristoyl phosphatidyl choline and distearoyl phosphoethanolaminepolyethylene glycol 2000 in a 95:5 molar ratio had a 6.5 nm size and loaded 6% CsA w/w. Despite lower loading compared to the block polymer micelle, the mixed micelles were able to incorporate twice as much CsA compared to a liposome. No significant differences in distribution were measured compared to the CrEL formulation (Lee et al., 1999).

Nanospheres and microspheres of poly(DL-lactide-co-glycolide) (1:1 molar ratio) were used to load CsA. The release rate of CsA was found to be biphasic. The first phase was characterize by a rapid release (initial burst), followed by a continuous and slower release thereafter. The length and release rate of the initial phase was defined by the size of the sphere. Microspheres of 30 μm had a slower release rate over 5 days with only 10–15% of the dose released. The nanospheres of 0.2–1 μm released 25–35% of the dose within the first day, likely as a result of their higher surface area per unit mass. However, in the second phase, the release rate was greater for the microspheres compared to that of the nanospheres. Sustained release of CsA took place over 28 days with 50% of the dose remaining.

POTENTIAL PROBLEMS AND LIMITATION

Narrow Therapeutic Window

CsA is a drug which has a very narrow therapeutic range as shown in Fig. 4. The therapeutic range is defined by the minimum concentration required to

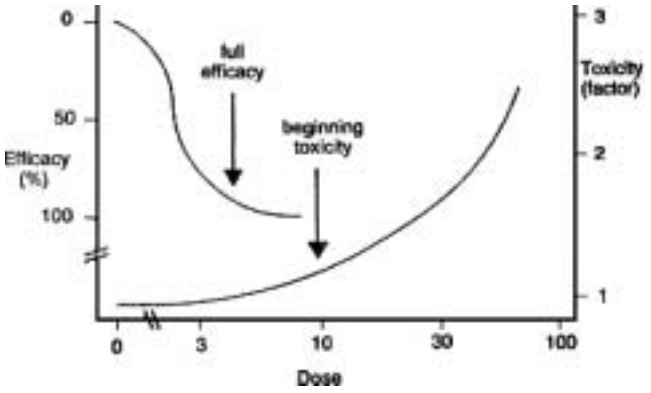

FIGURE 4 **Schematic Representation of the Narrow Therapeutic Window which Exist for CsA Between the Concentrations Required for Maximum Response and where Toxicity Occurs (Mason, 1992).**

obtain full efficacy and the concentration where toxicity starts to occur. Below the therapeutic range, the immune system may not be sufficiently suppressed leading to organ rejection. However, excessive levels of CsA can also lead to adverse events such as nephrotoxicity, hepatotoxicity, neurotoxicity, hypertension and dyslipidemia. The intra- and inter-individual variations in the bioavailability of oral CsA present a major challenge. Within a group of patients receiving the same oral dose of CsA, some may experience acute rejection, while others will experience toxicity events (Kahan, 2004).

Intra-individual Variability

Food can affect the bioavailability of CsA administered orally. The ingestion of grapefruit juice was shown to increase the bioavailability of CsA by 30 to 290%. Compounds present within the grapefruit juice likely inhibit cytochrome P-450A4, thereby reducing the metabolism of CsA (Bistrup et al., 2001). The effect of food intake on CsA absorption has been debated. With Sandimmune®, the Ptachcinski group found that the AUC of CsA increased on average by 61% when administered with food compared to without food. However, in 3 out of 18 patients, food intake did not alter absorption (Ptachcinski et al., 1985). The Honcharik group reported opposing results, whereas oral administration of CsA in a fasting state or with a breakfast containing trace or moderate fat content did not significantly alter the absorption of the drug (Honcharik et al., 1991). The Kahan group observed that diet did not affect the AUC of the Sandimmune® or Neoral® formulations (Kahan et al., 1995).

Other factors influencing intra-individual variability include the time elapsed since transplantation. Bioavailability can increase by 24–51% within 2 to 4 months following transplantation (Lindholm, 1991) .

Inter-individual Variability

Liver function can be variable in the patients receiving CsA. This may be attributed to disease or recovery following transplantation. This can result in reduced bile production and flow which would significantly affect absorption of CsA from the Sandimmune® formulation and may reduce bioavailability below 5% (Lindholm, 1991). As a result of its decreased dependence on bile, Neoral® absorption would be affected to a lesser extent, especially in liver transplant patients undergoing biliary diversion or with cholestasis (Noble and Markham, 1995). Altered liver function can also decrease the metabolism of CsA.

Gastrointestinal state can also vary between individuals receiving oral CsA. Diarrhea can decrease the absorption of CsA. Motility as well as the length of the small intestine may also lead to variable absorption.

Genetics can also play a role in the observed variability. Inherited variability in the concentration and/or structure of cytochrome P-450 can result in variable degradation in the liver and to a lesser extent in the enterocytes. The metabolizing activity of the liver was reported to vary by as much as 25-fold between individuals (Lindholm, 1991).

CONCLUSION

The vehicles used for the oral administration of CsA have evolved from crude emulsions (Sandimmune®) to microemulsions (Neoral®). Although the second generation of oral delivery vehicles provides greater bioavailability and is less dependent on bile secretions, there remains a significant variability between different individuals. This could be due in part to variable metabolism by cytochrome P-450 3A enzymes or the interaction with transporter such as P-glycoproteins present on the apical membrane of enterocytes and on the hepatocyte canalicular membrane. Alternative delivery systems show some promising characteristics. Liposomes appear to increased hepatic CsA concentrations despite their limited loading capacity. Oral micelles and nanoparticles

could increase the bioavailability of CsA to level approaching or exceeding that of Neoral®. Furthermore, when administered intravenously, micelles as wells as micro and nanoparticles could provide a sustained release rate of CsA over extended periods. However, an ideal delivery system for CsA exhibiting high loading capacity and bioavailability, targeted delivery to the site of the allograft following systemic administration and sustained release rates has yet to be designed.

REFERENCES

Alangary, A. A., Bayomi, M. A., Khidr, S. N., Almeshal, M. A., & Aldardiri, M. (1995). Characterization, stability and in-vivo targeting of liposomal formulations containing cyclosporine. *International Journal of Pharmaceutics*, *114*(2), 221–225.

Aliabadi, H. M., Mahmud, A., Sharifabadi, A. D., & Lavasanifar, A. (2005). Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of Cyclosporine A. *Journal of Controlled Release*, *104*(2), 301–311.

Andrysek, I. (2003). Impact of physical properties of formulations on bioavailability of active substance: current and novel drugs with cyclosporine. *Molecular Immunology*, *39*(17–18), 1061–1065.

Atkinson, K., Biggs, J. C., & Britton, K. (1982). Distribution and persistence of cyclosporin in human tissues. *The Lancet*, *320*(8308), 1165.

Bistrup, C., Nielsen, F. T., Jeppesen, U. E., & Dieperink, H. (2001). Effect of grapefruit juice on Sandimmun Neoral (R) absorption among stable renal allograft recipients. *Nephrology Dialysis Transplantation*, *16*(2), 373–377.

Bohme, M., Buchler, M., Mueller, M., & Keppler, D. (1993). Differential inhibition by cyclosporins of primary-active atp-dependent transporters in the hepatocyte canalicular membrane. *Febs Letters*, *333*(1–2), 193–196.

Borel, J. F. (1989). The cyclosporins. *Transplantation Proceedings*, *21*(1): 810–815.

Calne, R. Y., Thiru, S., McMaster, P., Craddock, G. N., White, D. J. G., Evans, D. B., Dunn, D. C., Pentlow, B. D., & Rolles, K. (1978). Cyclosporin A in patients receiving renal allografts from cadaver donors. *The Lancet*, *312*(8104), 1323–1327.

Canadian Pharmacists Association. (2006). *Compendium of Pharmaceuticals and Specialties*. Ottawa: Canadian Pharmacists Association.

Chen, G., Duran, G. E., Steger, K. A., Lacayo, N. J., Jaffrezou J. P., Dumontet, C., & Sikic, B. I. (1997). Multidrug-resistant human sarcoma cells with a mutant P-glycoprotein, altered phenotype, and resistance to cyclosporins. *Journal of Biological Chemistry*, *272*(9), 5974–5982.

Choice, E., Masin, D., Bally, M. B., Meloche, M., & Madden, T. D. (1995). Liposomal cyclosporine - comparison of drug lipid carrier pharmacokinetics and biodistribution. *Transplantation*, *60*(9), 1006–1011.

El Tayar, N., Mark, A. E., Vallst, P., Brunne, R. M., Testa, B., & van Gunsteren, W. F. (1993). Sovent-dependent conformation and hydrogen-bonding capacity of cyclosporin A: evidence from partition coefficients and molecular dynamics simulations. *Journal of Medicinal Chemistry*, *36*(24), 3757–3764.

Francis, M. F., Cristea, M., Yang, Y. L., & Winnik, F. M. (2005). Engineering polysaccharide-based polymeric micelles to enhance permeability of cyclosporin a across Caco-2 cells. *Pharmaceutical Research*, *22*(2), 209–219.

Frei, U., Taesch, S., & Niese, D. (1994). Use of sandimmun-neoral in renal-transplant patients. *Transplantation Proceedings*, *26*(5), 2928–2931.

Freise, C. E., Liu, T., Hong, K. L., Osorio, R. W., Papahadjopoulos, D., Ferrell, L., Ascher, N. L., & Roberts, J. P. (1994). The increased efficacy and decreased nephrotoxicity of a cyclosporine liposome. *Transplantation*, *57*(6), 928–932.

Fricker, G., Drewe, J., Huwyler, J., Gutmann, H., & Beglinger C. (1996). Relevance of p-glycoprotein for the enteral absorption of cyclosporin A: In vitro in vivo correlation. *British Journal of Pharmacology*, *118*(7), 1841–1847.

Friis, G. J. & Bundgaard, H. (1992). Kinetics of degradation of cyclosporine-A in acidic aqueous-solution and its implication in its oral absorption. *International Journal of Pharmaceutics*, *82*(1–2), 79–83.

Gelderblom, H., Verweij, J., Nooter, K., & Sparreboom, A. (2001). Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *European Journal of Cancer*, *37*(13), 1590–1598.

Hebert, M. F. (1997). Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Advanced Drug Delivery Reviews*, *27*(2–3), 201–214.

Honcharik, N., Yatscoff, R. W., Jeffrey, J. R., & Rush, D. N. (1991). The effect of meal composition on cyclosporine absorption. *Transplantation*, *52*(6), 1087–1089.

Hows, J. M. & Smith, J. M. (1983). In vitro stability of cyclosporin A. *Journal of Clinical Pathology*, *36*(6), 720–721.

Jacobson, P. A., Maksym, C. J., Landvay, A., Weiner, N., & Whitmore, R. (1993). Compatibility of cyclosporine with fat emulsion. *American Journal of Hospital Pharmacy*, *50*(4), 687–690.

Kahan, B. D., Dunn, J., Fitts, C., Vanburen, D., Wombolt, D. Pollack, R., Carson, R., Alexander, J. W., Choc, M., Wong, R., & Hwang, D. S. (1995). Reduced intersubject and intrasubject variability in cyclosporine pharmacokinetics in renal-transplant recipients treated with a microemulsion formulation in conjunction with fasting, low-fat meals, or high-fat meals. *Transplantation*, *59*(4), 505–511.

Kahan, B. D. (2004). Therapeutic drug monitoring of cyclosporine: 20 years of progress. *Transplantation Proceedings*, *36*(2), 378s–391s.

Karlsson, J., Fong, K. S., Hansson, M. J., Elmer, E., Csiszar, K., Keep, M. F. (2004). Life span extension and reduced neuronal death after weekly intraventricular cyclosporin injections in the G93A transgenic mouse model of amyotrophic lateral sclerosis. *Journal of Neurosurgery*, *101*(1), 128–137.

Lallemand, F., Perottet, P., Felt-Baeyens, O., Kloeti, W., Philippoz, F., Marfurt, J., Besseghir, K., & Gurny, R. (2005). A water-soluble prodrug of cyclosporine A for ocular application: A stability study. *European Journal of Pharmaceutical Sciences*, *26*(1), 124–129.

Lechuga-Ballesteros, D., Abdul-Fattah, A., Stevenson, C. L., & Bennett, D. B. (2003). Properties and stability of a liquid crystal form of cyclosporine - The first reported naturally occurring peptide that exists as a thermotropic liquid crystal. *Journal of Pharmaceutical Sciences*, *92*(9), 1821–1831.

Lee, M. K., Choi, L., Kim, M. H., & Kim, C. K. (1999). Pharmacokinetics and organ distribution of cyclosporin A incorporated in liposomes and mixed micelles. *International Journal of Pharmaceutics*, *191*(2), 87–93.

Lindholm, A. (1991). Factors influencing the pharmacokinetics of cyclosporine in man. *Therapeutic Drug Monitoring*, *13*(6), 465–477.

Mason, J. (1992). Cyclosporins past, present, and future. *Transplantation Proceedings*, *24*(4), 61–63.

Molpeceres, J., Aberturas, M. R., & Guzman, M. (2000). Biodegradable nanoparticles as a delivery system for cyclosporine: preparation and characterization. *Journal of Microencapsulation*, *17*(5), 599–614.

Mueller, E. A., Kovarik, J. M., Vanbree, J. B., Lison, A. E., & Kutz, K. (1994). Pharmacokinetics and tolerability of a microemulsion formulation of cyclosporine in renal-allograft recipients - a

concentration-controlled comparison with the commercial formulation. *Transplantation*, *57*(8), 1178–1182.
Mueller, E. A., Kovarik, J. M., Vanbree, J. B., Tetzloff, W., Grevel, J., & Kutz, K. (1994). Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharmaceutical Research*, *11*(2), 301–304.
Noble, S. & Markham, A. (1995). Cyclosporin - a review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (neoral). *Drugs*, *50*(5), 924–941.
Novartis_Phamaceuticals_Canada. (2004). *Prescribing Information: Neoral and Sandimmune I.V.*
Novartis_Phamaceuticals_Corporation. (2005a). *Neoral Soft Gelatin Capsules and Oral Solution*. Vol. T2005-23, 1–35.
Novartis_Pharmaceuticals_Corporation. (2005b). *Sandimmune Soft Gelatin Capsules, Oral Solution and Injection*. Vol. T2005-44.
Ohashi, R., Kamikozawa, Y., Sugiura, M., Fukada, H., Yabwchi, H., & Tamai, I. (2006). Effect of P-glycoprotein on intestinal absorption and brain penetration of antiallergic agent bepotastine besilate. *Drug Metabolism and Disposition*, *34*(5): p. 793–799.
Ouyang, C., Choice, E., Holland, J., Meloche, M., & Madden, T. D. (1995). Liposomal cyclosporine - characterization of drug incorporation and interbilayer exchange. *Transplantation*, *60*(9), 999–1006.
Pichard-Garcia, L., Whomsley, R., Davjat, M., Maurel, P., Setoyama, T., & Humphries, T. J. (2000). Comparative effects of rabeprazole and omeprazole on the inducibility of cytochrome P450-1A and cytochrome P450-3A isoenzymes in human hepatocytes, and effects on cyclosporin metabolism in human liver microsomes. *Clinical Drug Investigation*, *20*(4), 245–254.
Powles, R. L., Clink, H., Sloane, J., Barrett, A. J., Kay, H. E. M., & McElwain, T. J. (1978). Cyclosporin A for the treatment of graft-versus-host disease in man. *The Lancet*, *312*(8104), 1327–1331.
Ptachcinski, R. J., Venkataramanan, R., & Burckart, G. J. (1986). Clinical pharmacokinetics of cyclosporin. *Clinical Pharmacokinetics*, *11*, 107–132.
Ptachcinski, R. J., Venkataramanan, R., Rosenthal, J. T., Burckart, G. J., Taylor, R. J., & Hakala, T. R. (1985). The effect of food on cyclosporine absorption. *Transplantation*, *40*(2), 174–176.
Scheff, S. W. & Sullivan, P. G. (1999). Cyclosporin A significantly ameliorates cortical damage following experimental traumatic brain injury in rodents. *Journal of Neurotrauma*, *16*(9), 783–792.
Seeballuck, F., Ashford, M. B., & O'Driscoll, C. M. (2003). The effects of Pluronic (R) block copolymers and Cremophor (R) EL on intestinal lipoprotein processing and the potential link with P-glycoprotein in Caco-2 cells. *Pharmaceutical Research*, *20*(7), 1085–1092.
Sigma. (1996). *Cyclosporin A Product Information. C3667*, 1–2.
Varela, M. C., Guzman, M., Molpeceres, J., Aberturas, M. D., Rodriguez-Puyol, D., & Rodriguez-Puyol, M. (2001). Cyclosporine-loaded polycaprolactone nanoparticles: immunosuppression and nephrotoxicity in rats. *European Journal of Pharmaceutical Sciences*, *12*(4), 471–478.
Vonderscher, J. & Meinzer, A. (1994). Rationale for the development of Sandimmune-Neoral. *Transplantation Proceedings*, *26*(5), 2925–2927.
Yonan, N., Martyszczuk, R., Machaal, A., Baynes, A., & Keevil, B. G. (2006). Monitoring of cyclosporine levels in transplant recipients using self-administered fingerprick sampling. *Clinical Transplantation*, *20*(2), 221–225.
Yoshikawa, Y., Miyazaki, M., Hovjou, T., Komuta, Y., Yoshikawo, H., Takada, K. (1997). Biodistribution of cyclosporin encapsulated in liposomes modified with bioadhesive polymer. *Journal of Pharmacy and Pharmacology*, *49*(7), 661–668.
Zhang, Q. N., Yie, G. Q., Li, Y., Yang, Q. S., & Nagai, Y. T. (2000). Studies on the cyclosporin A loaded stearic acid nanoparticles. *International Journal of Pharmaceutics*, *200*(2), 153–159.