Chapter III

Cyclosporin

Cyclosporin (CsA) is a cyclic polypeptide of 11 amino acids isolated from the fungus *Tolypocladium inflatum gams*. It was discovered in 1972 in the screening test for antifungal activity. CsA was shown to have only weak antifungal activity but have a remarkable immunosuppressive properties, unique in selective effects on lymphocytes. The powerful immunosuppressive activity of CsA is recognized as a breakthrough in immunopharmacology, because it was the first agent that produce relatively specific immunosuppression, without causing bone marrow suppression. After the first clinical study in renal transplant recipients in 1978, CsA has become the drug of choice for the prevention of graft rejection. (Faulds, Goa, and Benfield, 1993)

Cyclosporin has many derivatives in addition to cyclosporin A (CsA), other derivatives are designated likewise such as cyclosporin B, C, D, etc. Each derivative is similar in their structure but different in their amino acid composition. The most powerful derivative is cyclosporin A (CsA). The chemical structure of CsA is shown in Fig 3.1. The peptide is neutral and rich in hydrophobic amino acids, this makes it poorly soluble in water (0.004% w/w) but soluble in lipids and other organic solvents.

Pharmacodynamic Properties

Cyclosporin acts relatively selective on T lymphocytes. The principle effect is to inhibit the transduction pathway for the synthesis of several cytokines from T-helper cells, especially interleukin-2 (IL-2). CsA forms a complex with cyclophilin, a cytoplasmic protein which is an intracellular receptor of CsA. Then this complex binds to calcineurin, a calcium-and calmodulin-dependent phosphatase. Inhibition of this phosphatase activity results in blocking the translocation of a nuclear factor, which is required for IL-2 transcription. The transcription of several other cytokines, including interferon-γ, and several other interleukin is also inhibited by CsA. IL-2 is necessary for activation and clonal expansion of T-cells and maturation of various other cell types. Consequently, as lymphokine synthesis and secretion

from T-Cells is inhibited, T-cell-dependent B cell responses will also be suppressed (Tsunoda, and Aweeka, 1996a)

CsA has been associated with vasoconstriction producing renal dysfunction and hypertension, though it does not have direct effect but appears to modulate the response to other substances in vascular beds. In kidney, CsA produces structural and functional changes affecting predominantly the proximal tubule and the afferent arteriole. These effects result in impaired renal function which is usually reversible and, less often, structural renal damage (Faulds, 1996).
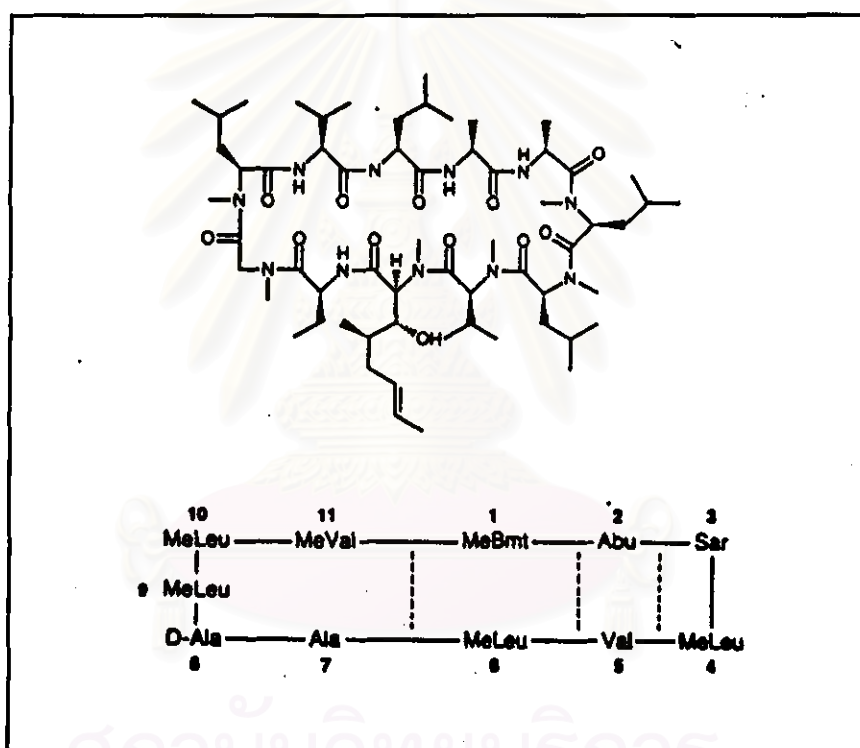

Figure 3.1 Structure of cyclosporin A

Pharmacokinetic Properties

Absorption

CsA absorption is slow, incomplete, and highly variable after oral administration. Bioavailability ranges from 2 to 89 % with a mean of 30%. Maximum concentrations are usually reached between 1 and 8 hours after oral dosing (Tsunodo and Aweeka, 1996a).

Absorption is influenced by many factors. The bioavailability of CsA appears to increase with time after transplantation. Bile is required for the absorption of CsA, thus factors affecting bile flow such as cholestasis and biliary diversion can decrease CsA absorption. Light meal has no effect on the absorption of CsA, whereas it might be enhanced by a fatty meal (Lindholm, 1991).

Drugs influencing gastric and intestinal motility may change CsA absorption. Metoclopramide, for example, shortened the time to peak concentration and increased the peak concentration, due to an enhanced rate of gastric emptying. The absorption of CsA is impaired in patients with diarrhea. Other dysfunction and severe disease of the gastrointestinal tract also decrease the CsA absorption (Fahr, 1993).

Distribution

Because of its highly lipophilicity, CsA is widely distributed throughout the body. In renal transplant recipients, the mean volume of distribution was 2.9 - 4.7 l/kg. The highest concentrations are found in fat and liver. It also sequesters in leukocyte-rich organs like thymus, spleen, and lymph nodes, and fatty organs such as pancreas and kidneys.

In blood, 58% of the circulating CsA is bound to erythrocytes, 9% to leukocytes, and 33% is in plasma (21% bound to lipoproteins, 8% to other plasma protein, and 4% in plasma water). Of the lipoproteins, high-density lipoprotein (HDL) binds 43-57% of CsA in plasma, low-density lipoprotein (LDL) binds 25% and very-low-density lipoprotein (VLDL) binds only 2% (Fahr, 1993).

The distribution of CsA between blood cells and plasma is highly temperature-dependent. The plasma separation at 37^0C gave 15% higher plasma concentrations of CsA than separation at 36^0C. However, the in vivo importance of this distribution phenomenon is unknown. The partitioning of CsA between blood and tissue compartments is affected by changes in hematocrit and lipoprotein concentrations, therefore anemia and lipid disorders may alter CsA pharmacokinetics (Awni et al., 1989).

The drug crosses the placenta and is presented in amniotic fluid and fetal circulation. It is also found in breast milk and breast-feeding should be avoided.

Metabolism and Elimination

CsA is extensively metabolized by cytochrome P450IIIA in the liver and gastrointestinal mucosa to more than 30 metabolites. The clinical significance of most of these metabolites remains unclear. The most active metabolite is AM1 (former nomenclature M17), with 10 to 20% of the immunosuppressive activity of parent CsA. Other less active metabolites are AM9 and AM4N.

Biliary excretion is the major route of elimination. More than 90% of CsA dose is excreted in bile with less than 1% excreted as unchanged CsA and greater than 40% appearing as metabolites. The urinary elimination is of minor importance. The urinary excretion was found to be 6% of an oral dose with less than 1% as parent drug. Thus, dosage adjustment in patients with renal insufficiency is not warranted; however, patients with hepatic failure exhibit decreased cyclosporin clearance (Faulds et al., 1993).

CsA metabolism is age-dependent with an increased clearance and decreased elimination half-life in children, who may have increased dosage requirements.

Drug interactions

Since cytochrome P450IIIA may be responsible for more than 50% of the metabolism of all drugs, the potential for drug interactions is immense. Some of these drug interactions are of clinical significance. Agents such as ketoconazole, erythromycin, diltiazem, and verapamil inhibit the cytochrome P450 system and when given concomitantly with CsA will decrease CsA metabolism and cause increased drug levels. On the other hand, agents such as phenytoin and rifampin induce the cytochrome P450 system, increasing the metabolism and decreasing drug levels. Although the most commonly reported mechanism is alteration of CsA metabolism, there is increasing evidence that erythromycin, metoclopromide, and probably other drugs increase the absorption of CsA.

In addition to the pharmacokinetic drug interactions, pharmacodynamic interactions may also occur when CsA is administered with certain therapeutic agents. Some drugs can potentiate the nephrotoxicity of CsA such as aminoglycosides, amphotericin-B. Other important interactions include potentiation of other toxicities of CsA such as minoxidil causing additive hirsutism, and nifedipine causing increased gingival hyperplasia (Campana et al., 1996; Yee and McGuire, 1990).

CsA itself may have significant effects on coadministered drugs. For example, CsA appears to inhibit the metabolism of HMG-CoA reductase inhibitors. Coadministration of lovastatin and CsA has been reported to result in rhabdomyolysis (Campana, et al., 1996; Yee and McGuire, 1990).

Cyclosporin-Sparing Agents

Drugs which affect CsA pharmacokinetics were initially seen as relatively contraindicated, but once the economic potential was realized, deliberate coprescription of drugs to allow a reduction in CsA dosage were soon advocated. The decision to choose these agents is also based upon the potential for additional therapeutic benefit and/or adverse effects. The drugs which have been studied in some detail for their CsA-sparing effect include calcium channel blockers (diltiazem and verapamil) and ketoconazole. Table 3.1 summarizes the potential benefits and risks associated with the use of diltiazem or ketoconazole as CsA-sparing agents (Jones, 1997).

Table 3.1 Potential benefits and risks associated with the use of diltiazem or ketoconazole as cyclosporin-sparing agents

Potential benefits	Potential risks
• Reduction in cyclosporin dosage (30% for diltiazem, >70% for ketoconazole) resulting in significant cost savings to the funding body	◊ Poorer compliance resulting in greater fluctuations in blood cyclosporin concentrations and therefore a greater risk of rejection
• Reduced renovascular resistance with consequent improved early graft function and better renal clearance (diltiazem)	◊ Adverse effect (including hypotension from diltiazem and hepatotoxicity from ketoconazole)
• Reduced incidence of hypertension (diltiazem)	◊ Increased risk of interactions with other drugs which may reduce efficacy, cause adverse effects and/or affect cyclosporin-sparing effect (primarily with ketoconazole)
• Reduced atherosclerosis (diltiazem)	◊ Unknown effects on cyclosporin metabolite concentrations with potential to alter the efficacy and/or toxicity profile of cyclosporin
• Reduced rates of infection (ketoconazole)	
• Augmented immunosuppression (diltiazem and ketoconazole)	

Calcium channel blockers are effective antihypertension in renal transplant recipients treated with CsA. Experimental data have shown possible synergistic immunosuppressive effects of calcium blocker used in conjunction with CsA. Moreover, calcium blockers have been reported to have beneficial effects on renal function in the post-transplant period. Diltiazem, verapamil and nicardipine have been reported to alter CsA levels. The postulated mechanism is inhibition of CsA metabolism through the cytochrome P450 system. Although prospective controlled studies have shown that concomitant diltiazem use reduce CsA dosage requirements by approximately 30%, the magnitude of the interaction declines over time and it does not occur in all patients. Likewise, there have been few controlled, prospective studies to confirm the reproducibility of the concomitant verapamil use.

Ketoconazole also reduces metabolism of CsA via cytochrome P450 system. The coprescription allows a reduction in CsA dosage of approximately 80%. The magnitude of this interaction increase with time, though interpatient variability in CsA dosage reduction also increase (Jones, 1997; Campana et al., 1996).

Adverse Effect

Unlike most other immunosuppressive agents, CsA has no depressant effects on the bone marrow. The most significant adverse effect of CsA is nephrotoxicity. CsA nephrotoxicity is dose and concentration dependent, involves a reduction in glomerular filtration rate, and is usually reversible on discontinuation. In renal transplant recipients, it is often difficult to differentiate between CsA-induced nephrotoxicity and allograft rejection (Tsunoda and Aweeka, 1996a).

Secondary toxicity directly related to or complicated by CsA nephrotoxicity includes hypertension, hyperkalemia, hyperuricemia and hypomagnesemia. Hypertension is a predominant clinical problem commonly encountered in most transplant patients, often requiring multidrug therapy. Other side effects are hirsutism, gingival hyperplasia, and a variety of neurologic syndromes such as headaches, tremor, and paresthesias can occur. Prevention and management of some common adverse events of CsA is shown in table 3.2 (Rossi et al., 1993). Less commonly reported adverse effects include hepatotoxicity, nausea, vomiting, allergic reactions, myalgias and arthralgias. Many of these were reported more frequently in the early cyclosporin experience due to the higher doses initially used in transplant.

Table 3.2 Adverse effects of cyclosporin

Adverse effects	Prevention/management
Hypertension	1. Dietary sodium restriction
	2. Calcium channel blocker[a]
	3. Second agent such as a β-blocker, ACE inhibitor, α-agonist, peripheral vasodilators
Hyperuricaemia	1. Dietary restrictions
	2. Urinary alkalinisation
	3. Allopurinol[b] or probenecid[c]
	4. Colchicine (acute gout attacks)[d]
Hyperkalaemia	1. Dietary restrictions
	2. Loop diuretics
	3. Sodium polystyrene sulphonate (if $K^+ > 6$ mmol/L)
Hyperlipidaemia	1. Dietary restrictions
	2. Steroid reduction or withdrawal
	3. HMG-CoA inhibitors
	4. Second-line agents (gemfibrozil, nicotinic acid, binding resins)[e]
Neurotoxicity	1. Correct electrolyte abnormalities
	2. Use oral formulation if possible[f]
Hypertrichosis	1. Avoid agents with similar toxicity (e.g. minoxidil)
Gingival hyperplasia	1. Oral hygiene
	2. Avoid other agents with similar toxicity (e.g. phenytoin, nifedipine)

a Diltiazem, verapamil and nicardipine inhibit the metabolism of cyclosporin.
b Reduce dose of concomitant azathioprine by 50 to 75% and monitor white blood cell count.
c Probenecid is only effective in patients with a creatinine clearance > 50 ml/min (3.0 L/h).
d Closely monitor white blood cell count in patients on colchicine and azathioprine.
e Toxicity of specific antilipid agents may be compounded in transplant patients.
Abbreviations: ACE = angiotensin converting enzyme; HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A reductase; K^+ = serum potassium.

Therapeutic Drug Monitoring

Monitoring of CsA concentration is an important part of CsA therapy and is done routinely in most transplant centers. Since CsA has a narrow therapeutic range and wide pharmacokinetic variation among and within individuals, tailoring therapy to a specific patient is difficult. In addition, the dose of CsA correlates poorly with blood concentration. Therefore, therapeutic drug monitoring is used to help guide therapy and make dosage adjustment in order to maximize drug efficacy while minimizing toxicity.

Analytical Method

There are two general types of assays: those selectively detecting only parent CsA, and those nonselectively measuring composites of CsA plus varying arrays of metabolites. Most assays available for monitoring CsA are selective for the parent drug, based on the established guidelines (Shaw et al., 1989; Kahan et al., 1990; Shaw et al., 1990; Oellerich et al., 1995). The four most common assays are ranked in order of specificity, precision, accuracy, and cost in table 3.3 (Sketris et al., 1995).

Table 3.3 Ranking of Cyclosporin Assays for Analytical Performance

	Specificity	Precision	Accuracy	Cost
HPLC	1	4	2	1
FPIA	4	1	1	2
RIA	3	3	4	4
EMIT	2	2	3	3

Key: 1 = Highest; HPLC = High performance liquid chromatography; FPIA = Fluorescent polarization immunoassay; RIA = Radioimmunoassay; EMIT = Enzyme multiplied immunoassay technique.

Although HPLC is the method with the highest specific for parent compound CsA and is the reference method, it has numerous practical disadvantages: methods are rarely standardized, making comparisons of CsA measurements between centers difficult, technical expertise is required, turnaround times are slow and equipment is expensive. Therefore, the use of HPLC has been reserved for using as experimental tool.

Specific RIA involves the use of specific monoclonal antibodies as the detector of CsA. The advantages of specific RIA over HPLC are that they are technically less demanding and have a faster turnaround time.

FPIA method eliminates the problems of using radioactive substance, uses a reproducible, automated format, requires little technician expertise, and has a rapid turnaround time.

EMIT has been introduced more recently. It exhibits the greatest selectivity for the parent drug. The major disadvantage is its limited working range. The highest calibration standard is 500 µg/l. With specimens above that concentration, it must be diluted before analysis (Rodighiero, 1989; Kivisto, 1992; Tsunoda and Aweeka, 1996b).

Matrix for concentration measurements

Whole blood with EDTA as anticoagulant is the most commonly recommended as the medium for analysis based on analytical reasons. The advantages of using whole blood rather than plasma are that it obviates the problem associated with sample separation and avoids temperature effect on CsA equilibration between blood cells and plasma. However, from a clinical perspective, no significant advantage of monitoring CsA in whole blood versus plasma has been reported (Shaw et al., 1987; Kahan et al., 1990; Shaw et al., 1990; Oellerich et al., 1995; Sketris et al., 1995).

Trough Concentration Monitoring

Currently, most centers use trough CsA concentration for routine monitoring therapy. Predose concentration measurement has several advantages: it gives a reliable and reproducible measure of the minimum steady state concentration, it can be performed in outpatients, and it is the most documented monitoring method. However, the concentration-effect relationships are often weak. Patients displaying trough level within a putative CsA therapeutic range are not always spared from either rejection or nephrotoxicity. Besides, trough concentrations are a poor guide to dosage adjustments (Grevel, Welsh, and Kahan, 1989; Lindholm and Sawe, 1995).

Although trough level is generally monitored, there is no universal therapeutic range. The major limitation to defining the therapeutic ranges is the lack of standards for diagnosing toxicity or degree of efficacy because CsA is usually used for prevention of rejection. There is no readily marker that detects the degree of immunosuppression by CsA other than acute rejection by a tissue biopsy. The only readily available marker for nephrotoxicity has been serum creatinine level, which is insensitive to small changes in glomerular filtration rate. It is difficult to establish the therapeutic range of CsA since clinical studies have often use different analytic methods, different sample matrices (serum, plasma, or whole blood), different dosing schedules (twice daily, once daily), or different concurrent immunosuppressive agents in the various patient groups (table 3.4) (Oellerich et al., 1995).

Table 3.4 Therapeutic ranges for CsA of some transplant centers during 1994/1995

Center	Analytic method	Dosing interval	Immuno-suppression protocol	Therapeutic ranges (μg/L)	Transplant type
University of Pennsylvania Medical Center Philadelphia,PA, USA	HPLC	b.i.d.	III	100-250<3 mo 80 -125>3 mo	K
			III	200-300	L
			III	200-300<12 mo	H
			III	100-150>12 mo	
			III	250-350 <12 mo	Lu
			III	200-300>12 mo	
St. Christophers Hospital for Children, Philadelphia, PA, USA	m^{125}I-RIA	b.i.d., t.i.d.	III	100-200<3 mo 75-150 >3 mo	K
			III;I, 12-18mo	250-350<3 mo 150-250>3 mo	L
			III	250-350< 3 mo 100-200>3 mo	H
St. Vincent's Hospital, Darlinghurst, NSW, Australia	mFPIA	b.i.d.	III	250-375<6 mo 100-250>6 mo	K
			III	350-450>2 mo 300-400 2-3 mo 250-300 3-6 mo 200-300 6-12 mo 150-200> 12 mo	H, H-Lu
Neues, Allgemeines Krankenhaus, Vienna, Austria	mFPIA	b.i.d.	II	125-250<3 mo 100-200>3 mo	K
			II + ALA	125-250	L
Northwestern University Medical School, Chicago, IL, USA	mFPIA	b.i.d.	III	250-400<6 mo 250-300<6 mo	K
	HPLC	b.i.d.	IV	200 <6 mo	L
				200 <6 mo	Panc
Papworth Hospital, Cambridge, UK	EMIT	b.i.d.	III	300-400<3 mo 200-300 3-12 mo 100-250>12 mo	H
		b.i.d.	III	300-500<3 mo 200-300 3-12 mo 100-250>12 mo	H-Lu
King's College, London, UK	mFPIA	b.i.d.	III	150-250<3 wks 100-200 3-8 wks <125 >12 wks	Adult L
		b.i.d.	III	150-250<6 mo 100-150 6-12mo 50-100>12 mo	Ped L

I, CsA monotherapy; II, "double therapy": CsA + prednisone; III, "triple therapy": CsA + prednisone + azathioprine; IV, "quadruple therapy," induction therapy in which anti-lymphocyte antibody (ALA) is used as part of initial immunosuppression until good kidney function is achieved within 14 days after surgery.; K, kidney: H, heart: L, liver; Lu, lung: Panc, pancreas; Ped, pediatric.

Monitoring Area Under the curve

Because trough concentration may not reflect the total amount of drug exposure during the dosing interval, some centers advocate that individualized oral doses be based on the AUC measurement as a more useful tool to predict the clinical events (Kasiske et al., 1988; Grevel, Napoli et al., 1991; Grevel and Kahan, 1991a; Grevel and Kahan, 1991b; Lindholm and Kahan, 1993).

One of the obvious practical disadvantages of AUC monitoring is that numerous and frequent blood samples must be taken at the precisely time intervals. In attempt to minimize this, several studies have suggested abbreviated kinetic profiles (Johnston et al., 1990; Grevel and Kahan, 1991b, Meyer et al., 1993; Lindholm et al., 1993; Serino et al., 1994; Foradori et al., 1995; Tsang et al., 1996; Serafinowicz, Gaciong, Baczkowska et al., 1996).

The limited sampling strategy offers a practical solution to AUC monitoring while still providing more information than a single trough level measurement. However, this method cannot account for pharmacodynamic variability in patient response, nor can it consistently account for the pharmacokinetic changes which occur within an individual over time. Despite this, the AUC monitoring method is promising and may add to the other clinical information used for monitoring CsA therapy.

New microemulsion formulation

The conventional oral formulation of CsA is available as an oil-based solution and as soft gelatin capsules. After oral administration, a crude oil-in-water droplet mixture is formed on contact with gastric fluids. Bile salts is required for emulsification this mixture before digestion of the oily droplets and release of CsA can occur. This emulsification step makes the absorption of CsA depend on food intake, bile flow and gastrointestinal motility, and therefore unpredictable and markedly variable.

In an effort to improve the considerable variability in pharmacokinetic, a new formulation of CsA has been developed. It is a microemulsion preconcentrate which consists of the drug in a lipophilic solvent and a hydrophilic solvent, together with a surfactant. On contact with gastric fluids, it readily forms a homogeneous microemulsion, which is able to release CsA quickly, allows more complete absorption (Noble and Markham, 1995; Ritschel, 1996; Kovarik, Mueller, and Niese, 1996).

The new microemulsion of CsA has shown an increased rate and extent of drug absorption with lower inter-and intraindividual pharmacokinetic

variability when compared with the conventional formulation (Kovarik et al., 1994; Holt et al., 1995; Kahan et al., 1995). The influence of a fat-rich meal on CsA microemulsion pharmacokinetics was comparatively less than conventional formulation (Mueller, Kovarik, van Bree, Grevel et al., 1994).