BEN H. BROUHARD, MD, EDITOR

Cyclosporine: mechanisms of action and toxicity

ROBERT M. GRAHAM, MD

- **BACKGROUND** Newer immunosuppressive agents are permitting transplantation to be performed more frequently.
- **OBJECTIVES** To review the mechanisms of action and toxicity of cyclosporine.
- **DISCUSSION** Graft rejection is mainly cell-mediated, although a humoral (antibody) response may also be involved. Cyclosporine and related agents such as FK-506 and rapamycin selectively inhibit adaptive immune responses by blocking T cell-dependent biosynthesis of lymphokines, particularly interleukin 2 at the level of messenger ribonucleic acid (mRNA) transcription. Because cyclosporine is metabolized in the liver by P-450 enzymes, drugs that affect the P-450 system also affect the metabolism of cyclosporine. Hypertension is the most common side effect of cyclosporine. Cyclosporine-induced "nephrotoxicity" may be functional rather than anatomic, caused primarily by preferential constriction of the afferent renal arteriole. Bacterial and fungal infections are less common with regimens of cyclosporine plus prednisone than with azathioprine plus prednisone. Nevertheless, cyclosporine-treated patients are vulnerable to viral infections.
- **CONCLUSIONS** Although cyclosporine and related compounds represent an improvement over earlier immunosuppressive agents, they produce serious side effects with which the practitioner should be familiar.

■ INDEX TERMS: CYCLOSPORINE; TRANSPLANTATION; IMMUNOSUPPRESSION
■ CLEVE CLIN J MED 1994; 61:308–313

From The Victor Change Cardiac Research Institute, St. Vincent's Hospital, Sydney, Australia.

Address reprint requests to R.M.G., The Victor Change Cardiac Research Institute, St. Vincent's Hospital, Darlinghurst 2010, Sydney, New South Wales, Australia.

TRANSPLANTATION of organs or bone marrow is widely used in organ-system failure and malignant diseases. The frequency with which transplantations are performed depends upon the availability of suitable alternatives to transplantation (eg, dialysis for end-stage renal disease), the availability of donor organs, the ability to prevent or treat rejection reactions, and the amount of damage that the graft can tolerate and still function effectively. The most successful transplantation programs have been with kidneys and corneas. However, now that more effective immunosuppressive drugs are available, transplantation of bone marrow and other organs is also performed frequently. This review will focus on the mechanisms of action and toxicity of these newer immunosuppressive agents, particularly cyclosporine.

CYCLOSPORINE

Cyclosporine, a neutral, lipophilic, cyclic undecapeptide with a molecular weight of 1203, is derived from the fungus *Trichoderma polysporin*. The amino acids in positions 1, 2, 3, and 11 form a hydrophobic

active immunosuppressive site. All natural and synthetic congeners with substitutions or deletions on the ring structure have less immunosuppressive activity than does cyclosporine.

Because of its lipophilicity, cyclosporine is stabilized with castor oil for intravenous administration or with olive oil for oral administration. The relative bioequivalence of oral to intravenous doses is 1:3. To avoid drug interactions with plasticizers, cyclosporine is dispensed from glass bottles. Constant 24-hour infusions are associated with fewer toxic reactions than bolus injections, but for convenience many physicians administer a 4-hour infusion. Oral doses of cyclosporine are usually given once or twice daily, depending on the plasma or blood levels and on renal function. Ophthalmic formulations produce high drug levels in the cornea, sclera, and lacrimal glands, prolong the survival of corneal allografts, and ameliorate ocular inflammatory disorders. Topical preparations produce favorable responses in patients with chronic dermatitis, but oral preparations are required to control skin-graft rejection and psoriasis. In addition, cyclosporine has been used in the treatment of systemic lupus erythematosus, myasthenia gravis, adult-onset and juvenile diabetes, nephropathies of the minimal-change, focal, segmental, membranous, and immunoglobulin A (IgA) types, schistosomiasis, and malaria.

Cyclosporine is metabolized by hepatic cytochrome P-450 enzymes; concomitant administration of drugs that interact with the P-450 system may affect cyclosporine's metabolism (*Table 1*). Elimination is by hepatic metabolism and biliary excretion. Most circulating cyclosporine is associated with lipoproteins of high, low, or very low density, and with chylomicrons. Some cyclosporine circulates freely, but this fraction does not correlate with the total blood level or with adverse clinical events. Toxic effects on the central nervous system are enhanced if the serum cholesterol concentration is less than 120 mg/dL.

FK-506 and rapamycin are macrolide antifungal agents derived from *Streptomyces tsukubaensis* and *Streptomyces hygroscopicus*, respectively. These agents produce immunosuppressive effects similar to those of cyclosporine, although via different effectors and pathways. They effectively block allograft rejection at doses 10 to 100 times lower than those required for cyclosporine. However, clinical experience with these agents is still limited.

TABLE 1
DRUGS THAT AFFECT CYCLOSPORINE LEVELS

Increase cyclosporine levels (inhibit cytochrome P-450)
Ketoconazole
Erythromycin
Oral contraceptives
Calcium antagonists (diltiazem > verapamil > nifedipine)
Decrease cyclosporine levels (induce cytochrome P-450)
Rifamycin
Phenobarbital
Phenytoin
Carbamazepine
Valproate

HOW CYCLOSPORINE WORKS

After they emerge from the bone marrow, T-cell precursors migrate to the thymus, where they mature. They then disseminate throughout the body, selectively binding antigens through specific receptors. This immune recognition "primes" T cells to express surface receptors for lymphokines, which are humoral immune transmitters that trigger cellular maturation. A second series of T-cell recognition reactions, the activation cascade (*Figure*), results in the synthesis of lymphokines and the acquisition of cytotoxic potential.

Cyclosporine does not affect the priming reaction. T cells stimulated in the presence of therapeutic doses of cyclosporine can still express receptors for lymphokines such as interleukin 2 (IL-2) and also become primed for B-cell stimulation, antiviral delayed-type hypersensitivity, and alloantigenic responses. Cyclosporine also does not affect the initial plasma-membrane delimited T-cell receptor events of signal reception, transduction, and calcium influx and has no effects on marrow-colony formation or on lymphokine-dependent proliferation.

Instead, cyclosporine primarily inhibits the activation cascade necessary for specific immune functions by blocking T-cell-dependent biosynthesis of lymphokines, particularly IL-2 (*Figure*). Thus, cyclosporine blocks IL-2 gene expression at the level of messenger ribonucleic acid (mRNA) transcription. Other effects of cyclosporine include actions at the level of the thymus to abort intrathymic T-cell development, and the inhibition of some T-cell-independent B-cell responses.

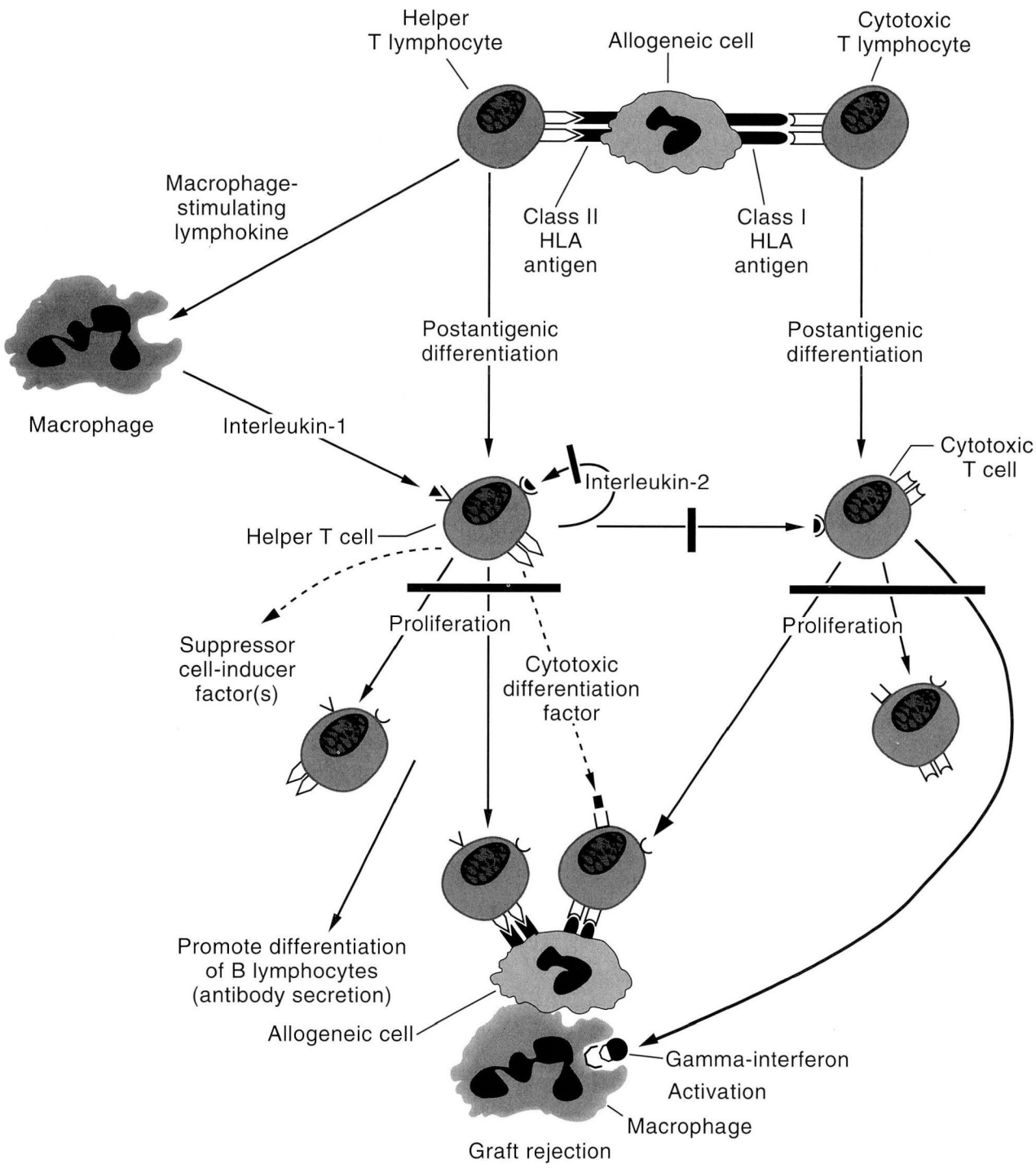

FIGURE. Effect of cyclosporine on the rejection cascade associated with allogeneic transplantation. Helper T cells recognize allogeneic antigens, but cyclosporine blocks production of interleukin-2; hence, this cell and other T cells fail to receive the signal to divide. Adapted with permission from an illustration by Bunji Tagawa in Strom TB, Carpenter CB. Renal transplantation: immunogenetic and clinical aspects—part II. Hospital Practice 1983; 18:135–150.

TABLE 2
IMMUNOPHILINS

Immunophilin	Organisms
Cyclophilins A, B, and C	Mammals
Cyclophilins 1 and 2	*Neurospora crassa*
Cyclophilins	Prokaryotes
Nina A protein	*Drosophila*
FK-binding proteins	Mammals
FK-binding proteins	Yeast
FK-binding proteins	*Neisseria meningitidis*
S-cyclophilin	Chickens
Secreted cyclophilin-like protein	Humans

Recent studies have provided considerable insights into the precise mechanism by which cyclosporine produces its immunosuppressive effects. Cyclosporine does not activate plasma-membrane receptors. Instead, cyclosporine, because of its lipophilic nature, readily and passively diffuses into cells, where it binds to a cytosolic protein or immunophilin, cyclophilin. This 17-kd protein displays a high affinity for immunosuppressive analogues of cyclosporine and a low affinity for inactive analogues.

The role of cyclophilin has been called into question because it is expressed in all cells and tissues, and it is only one member of an increasingly diverse family of general and tissue-specific cyclophilins (*Table 2*). However, a new member of this family, cyclophilin C, has recently been cloned and characterized. This protein shows extensive homology with all previously identified cyclophilins. It is expressed in fewer tissues than cyclophilins A and B but is present in those tissues most affected by cyclosporine.

SIDE EFFECTS OF CYCLOSPORINE

Table 3 lists the major side effects of cyclosporine. Immunologic complications such as bacterial and fungal infections occur less frequently with regimens of cyclosporine plus prednisone than with azathioprine plus prednisone. This is probably because lower dosages of steroids are required with cyclosporine. Nevertheless, cyclosporine-treated patients acquire viral infections, especially with herpes simplex, herpes zoster, and cytomegalovirus. Additionally, these patients may be at risk of acquiring

TABLE 3
SIDE EFFECTS OF CYCLOSPORINE*

Side effect	Incidence (%)
Hypertension	20 to 95
"Nephrotoxicity" (serum creatinine > 2.0 mg/dL)	< 30
Hepatotoxicity (abnormal liver enzymes)	10
Tremor	20
Hirsutism	20
Gingival hypertrophy	20
Viral infections (immunosuppression)	25
Anorexia	20
Hyperuricemia	80
Gout	10
Paresthesia or hyperesthesia	35
Hyperkalemia (serum potassium > 5.5 mEq/L)	95
Hypomagnesemia (serum magnesium < 1.2 mEq/L)	20

*Data courtesy of Ron Victor (see acknowledgment)

Pneumocystis carinii pneumonia.

Cyclosporine has little effect on either basal or stimulated function of natural killer cells that mediate immunosurveillance, which may explain why cyclosporine, at least in animal studies, does not promote tumor induction or mutagenicity either in vivo or in vitro. Abnormal liver-function tests and serum electrolyte concentrations are seen but are of limited clinical significance. Hyperkalemia often is related to the development of hyporeninemic hypoaldosteronism (type IV renal tubular acidosis), which is presumably due to the effects of cyclosporine on the renal tubule. Tremor and paresthesias are the most common forms of neurotoxicity. In addition, seizures related to malignant hypertension have been reported in children. Cyclosporine-induced hyperuricemia leads to gout in fewer than 10% of patients. Importantly, the majority of these side effects reverse upon discontinuation of cyclosporine.

Hypertension

Hypertension is the most common side effect of cyclosporine. In heart-transplant recipients the frequency of hypertension approaches 95%, whereas in some bone marrow-transplant recipients and patients with myasthenia gravis treated with cyclosporine, the frequency is 50% to 60%. Children may be more sensitive than adults to the hypertensive effects of cyclosporine. However, 5- to 10-fold

higher doses are used in children to achieve "therapeutic" levels because children have a high rate of hepatic metabolism. Early studies suggested that cyclosporine-induced hypertension is caused by direct toxic effects on the kidney. However, this may not be entirely accurate.

Nephrotoxicity

Several syndromes of cyclosporine-induced nephrotoxicity have been described. Asymptomatic, dose-dependent and reversible decreases in the glomerular filtration rate (GFR) probably occur to some extent in most cyclosporine-treated patients. Fortunately, cyclosporine rarely causes progressive destruction of functioning nephrons leading to end-stage renal failure. The incidence of end-stage renal failure was about 0.5% in earlier series using doses that were more than two times higher than those used currently. A syndrome resembling hemolytic-uremic syndrome or acute thrombotic thrombocytopenic purpura characterized by acute thrombotic occlusion of the renal microcirculation with multiple organ involvement seems to be an idiosyncratic reaction that also occurs very rarely.

Earlier anatomic studies focused on the tubular epithelium as the primary site of cyclosporine-induced renal injury, but this concept has been very difficult to establish for several reasons. First, the histologic lesions are rather nonspecific. Second, most but not all of the histologic studies in humans have been performed on biopsy specimens from renal transplant recipients, in whom it is very difficult to distinguish cyclosporine toxicity from transplant rejection. Third, it has been extremely difficult, if not impossible, to produce renal lesions by giving cyclosporine to experimental animals.

These findings have prompted the conclusion that renal damage per se does not contribute importantly to the development of cyclosporine-induced hypertension. This conclusion is strongly supported by a wealth of clinical data showing that, in the vast majority of patients, elevation in arterial blood pressure is *dissociated* from reduction in estimated GFR. Hypertension often occurs with minimal reductions in GFR, and there is no significant difference in GFR between patients who become hypertensive and those who remain normotensive during long-term administration of cyclosporine.

Recent studies have provided increasing evidence that cyclosporine-induced "nephrotoxicity" is functional rather than anatomic and is caused primarily by preferential constriction of the afferent renal arteriole. Numerous studies in both patients and rats have shown that cyclosporine-induced reduction in GFR is universally accompanied by decreases in renal blood flow.

How cyclosporine affects renal vascular resistance

A key question, therefore, is what mechanisms cause the increases in renal vascular resistance, particularly preglomerular resistance, evoked by cyclosporine? The answer to this question may provide important clues about the mechanism by which cyclosporine raises blood pressure.

Renin-angiotensin system. Numerous investigators have considered the possibility that activation of the renin-angiotensin system may be the cause (as well as the consequence) of the increased renal vascular resistance and hypertension evoked by cyclosporine. This is very unlikely for several reasons.

First, although plasma renin activity (PRA) clearly increases when cyclosporine is given for a short time to rats, several studies have demonstrated that this response is usually transient. With long-term administration of cyclosporine, PRA often tends to return to, or even falls below, the control value, an effect that is usually explained by a gradual increase in plasma volume. Again, the interpretation of many of the rat studies is complicated by the use of high doses, which made the rats sick and caused them to lose weight.

Second, long-term treatment with cyclosporine in humans consistently has been accompanied by diminished PRA. In cyclosporine-treated patients, PRA fails to increase appropriately both during orthostatic stress and during beta-adrenergic stimulation.

Finally, converting-enzyme inhibition has little or no effect on the renal hemodynamic responses to long-term administration of cyclosporine in rats or on blood pressure and PRA in cyclosporine-treated heart-transplant recipients.

Thus, long-term administration of cyclosporine in humans appears to decrease, not increase, the activity of the renin-angiotensin system. Experimental evidence suggests that cyclosporine inhibits the release of renin by juxtaglomerular cells and impairs the ability of angiotensin II to stimulate the release of aldosterone. There is increasing evidence that angiotensin II has important effects on intrarenal and systemic hemodynamics; in particular,

angiotensin II causes comparable constriction of afferent and efferent arterioles. "Down-regulation" of the renin-angiotensin system, therefore, prompts the hypothesis that cyclosporine-treated patients may lack effective means of maintaining the proper balance between preglomerular and postglomerular resistances, a role generally served by intrarenal angiotensin II.

Prostaglandins. Although cyclosporine alters prostaglandin secretion, no evidence supports the notion that prostaglandins are involved in the initiation or maintenance of the elevated vascular resistance and arterial pressure during long-term cyclosporine therapy.

Sympathetic nervous system. There is now abundant and direct evidence that the major mechanism for cyclosporine-induced hypertension is an increase in sympathetic nerve activity due both to a direct effect of cyclosporine and, in heart transplant patients, to a lack of ventricular baroreceptor restraint on sympathetic outflow, resulting from the denervation of the donor heart.

Increased sympathetic outflow raises blood pressure by causing vasoconstriction, particularly in the kidney, and by promoting salt and water retention through activation of proximal tubular alpha-adrenergic receptors. This latter effect explains the progressive increases in plasma volume associated with cyclosporine-induced hypertension.

Effects on vascular smooth muscle. In addition to stimulating central sympathetic outflow, cyclosporine also has been shown to enhance the effects of norepinephrine and other vasoconstrictors on vascular smooth muscle.

Cellular mechanisms of cyclosporine-induced hypertension. The cellular mechanisms underlying the direct effects of cyclosporine to enhance sympathetic nerve activity are presently unknown. However, cyclophilin is also present in high concentrations in nonlymphoid tissues such as the brain and kidney, target organs that may account for cyclosporine's effect on sympathetic nerve activity.

Interestingly, preliminary data from the intravenous administration of cyclosporine, FK-506, and analogues in rats have demonstrated marked increases in sympathetic nerve activity and blood pressure with cyclosporine and FK-506, lesser increases with less-immunosuppressive FK-506 analogues, and no increase with inactive FK-506 analogues or, interestingly, with rapamycin.

SUMMARY

The introduction of cyclosporine in clinical practice in the United States in the 1980s has had a major impact on organ transplantation and on therapy of other diseases. However, with increasing use came the recognition that this immunosuppressant had potentially severe side effects. We have begun to make significant progress in understanding the cellular and molecular mechanisms of these side effects. Other immunosuppressants have also been used in transplantation and are under investigation. Some agents (eg, FK-506) have actions similar to cyclosporine; others (eg, rapamycin) act synergistically with cyclosporine. As more patients are being evaluated for organ transplantation or organ donation, busy practitioners must familiarize themselves with the actions and side effects of these immunosuppressants and their interactions with other commonly used drugs.

ACKNOWLEDGMENTS

I am grateful to Ron Victor, University of Texas Southwestern Medical Center, Dallas, for allowing me to look over his excellent monograph, "The enigma of cyclosporin-induced hypertension," and to Ms. J. Holl for typing this manuscript.

BIBLIOGRAPHY

Kahan BD. Drug therapy: cyclosporine. N Engl J Med 1989; **321:**1725–1737.

Mark A. Cyclosporine, sympathetic activity and hypertension. N Engl J Med 1990; **323:**748–752.

McKeon F. When words collide: immunosuppressants meet protein phosphatases. Cell 1991; **66:**823–826.

Schreiber SL. Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science 1991; **251:**283–287.

Segal NH, Sickierka JJ, Dumont FJ. Observations on the mechanism of action of FK-506. A pharmacologic probe of lymphocyte signal transduction. Biochem Pharmacol 1990; **40:**2201–2208.

Skorecki KL, Rutledge WP, Schreer RW. Create cyclosporine nephrotoxicity - prototype for a renal membrane signalling disorders. Kidney Int 1992; **42:**1–10.