12 Cyclosporins: Recent Developments in Biosynthesis, Pharmacology and Biology, and Clinical Applications

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1 Introduction

Cyclosporins are a family of hydrophobic cyclic undecapeptides with a remarkable spectrum of diverse biological activities. The first member of this class to be discovered was named cyclosporin A (CsA; structure shown in Fig. 1). To this date, some 30 members of this family have been isolated from natural sources. In addition, a close analog named SDZ 214-103, incorporating a lactone function in place of a peptide bond and exhibiting a similar biological profile was also discovered from natural sources (Fig. 2). The discovery and structure elucidation of cyclosporins have been amply reviewed (WENGER 1986; WENGER et al., 1986; VON WARTBURG and TRABER, 1988; FLIRI and WENGER, 1990).

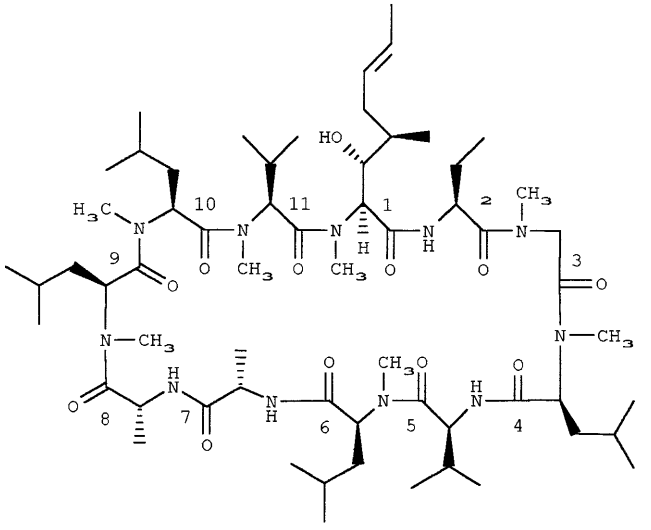

Fig. 1. Structure of cyclosporin A (Sandimmun®), including the numbering system.

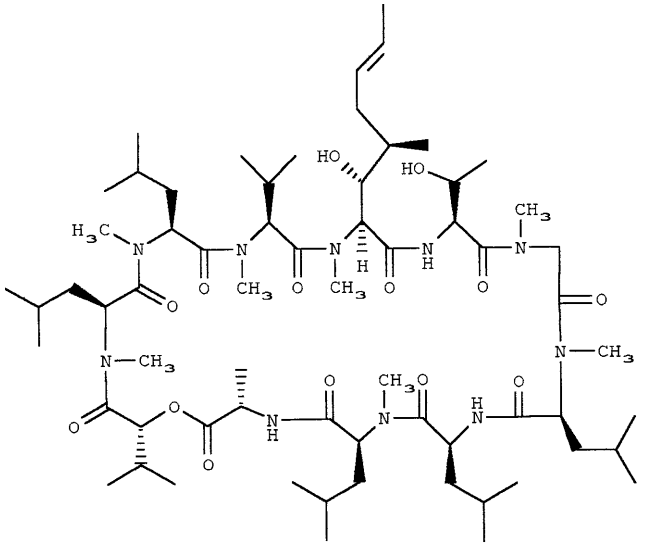

Fig. 2. Structure of SDZ 214-103.

In this chapter, emphasis is given to some more recent aspects of chemistry, biosynthesis, and biological activity.

2 Clinical Applications of Cyclosporins

2.1 Introduction

Cyclosporin A was initially isolated as an antifungal antibiotic. It was later shown to possess immunosuppressive properties of high therapeutic value. Since 1983, cyclosporin A, under the trade name Sandimmun®, has been in clinical use worldwide to prevent rejection of organ transplants. It has subsequently been approved for the therapy of certain autoimmune diseases. Since the time of market introduction of Sandimmun®, many additional biological activities of cyclosporins have been discovered, some of which may lead to novel clinical applications of cyclosporin A or of non-immunosuppressive analogs. At the time of market introduction of Sandimmun®, the mechanism by which this drug mediates immunosuppression was not understood at the molecular level nor was a receptor known. Since then, not only was a whole family of receptors discovered (i.e., the cyclophilins), but a possible role of these proteins for protein folding and cellular protein traffic has emerged. Much of what is known today about cyclosporins, cyclophilins and their biochemistry was greatly aided by the discovery of FK506, an immunosuppressive macrolide. This compound elicited much interest because, like cyclosporin A, it was a T cell selective immunosuppressant, but much more potent. This activity was soon shown to be based on a mechanism identical to that of cyclosporin. Search for FK506 receptors led to the discovery of the FK506 binding proteins (FKBPs), a novel protein family with no homologies to cyclophilins, yet with many properties in common. Like the cyclophilins, FKBPs appear to have a functional role in protein folding. Unveiling the mechanism of immunosuppressive activity of Sandimmun® and FK506 was greatly facilitated by the availability of a third compound, rapamycin, which binds to the same receptors as FK506, yet exhibits a different spectrum of biological activities. A detailed account of these aspects is given in Sects. 3.2. and 3.3. In this section present clinical applications of Sandimmun® are discussed. They include the following indications:

– allograft rejection,
– Behset's uveitis,
– rheumatoid arthritis,
– aplastic anemia (NDA's pending),
– nephrotic syndrome,
– atopic dermatitis (NDA's pending),
– psoriasis vulgaris.

2.2 Transplantation

Sandimmun® is a reversible inhibitor of the transcription of interleukin 2 (IL-2) and several other lymphokines, most notably in helper T lymphocytes (see Sect. 3.2). As a consequence, it suppresses the activation and/or maturation of various cell types, in particular those involved in cell-mediated immunity. Because of these properties, Sandimmun® has become the first-line immunosuppressant for prophylaxis and therapy of transplant rejection. In fact, the modern era of transplantation surgery was only possible after the availability of cyclosporin. The first patient to receive a kidney graft under CsA treatment was reported in 1978 (CALNE et al., 1978). Soon thereafter, transplantations of liver, heart, and combined lung–heart commenced (ERNST, 1991; BARRY, 1992; KAHAN, 1992; TSANG et al., 1992). In 1991, only in Germany 450 liver transplantations were performed (HOPF et al., 1992). Organ availability has become a major limiting factor and numerous patients die while awaiting a donor organ. Therefore, organ preservation techniques have become an important aspect in the area of transplantation surgery. Histocompatibility matching, besides immunosuppression, is the key factor contributing to long-term graft survival. Currently, expected 10-year first graft survival rates for kidneys from HLA-identical siblings, 1-haplotype-matched relative, and cadaver donors are 74, 51, and 40%, respec-

tively (BARRY, 1992). The survival probability after lung transplantation is approximately 65% after 1 year and 50% after 3 years (ERNST, 1991). Major problems encountered in transplantation surgery are technical difficulties during operation, serious infections, and acute rejection episodes during the first postoperative period, and chronic (long-term) rejection. Side effects can be classified into those associated to immunosuppression (lymphoproliferative disorders, infectious diseases caused by bacterial and fungal pathogens as well as viruses), and other adverse effects which are specific for the immunosuppressive drugs used. For Sandimmun® these include primarily impairment of renal function, hypertension, hirsutism, and gingival hyperplasia (MASON, 1989). Neurological and gastrointestinal effects are also common in Sandimmun® recipients but are usually mild to moderate and resolve on dosage reduction.

2.3 Autoimmune Diseases

Since Sandimmun® not only suppresses cell-mediated immunity but also humoral immune responses and inhibits chronic inflammatory reactions it appeared very promising in the treatment of autoimmune diseases. Prospective controlled trials performed in patients with autoimmune diseases have recently been reviewed (FREY, 1990; FAULDS et al., 1993). Efficacy could be proven for the following diseases: Endogenous uveitis, rheumatoid arthritis, Sjogren's syndrome, myasthenia gravis, psoriasis, atopic dermatitis, Crohn's disease. The drug is considered as a first-line therapy in patients with moderate or severe aplastic anemia who are not eligible for bone marrow transplantation. It may also be of benefit in patients with primary biliary cirrhosis and intractable pyoderma gangrenosum. Sandimmun® does not appear to be effective in patients with allergic contact dermatitis, multiple sclerosis, or amyotropic lateral sclerosis. Successful application in insulin-dependent diabetes will depend on the development of diagnostic tools indicating early disease onset before beta cell destruction has progressed too far and clinically overt diabetes is present. The most significant advantage in a number of indications is the steroid-sparing effect of Sandimmun®. To avoid relapse after control of active disease, patients should continue receiving Sandimmun® maintenance therapy at the lowest effective dose.

2.4 Activity Against Tumor Multidrug Resistance

Cellular resistance to cytotoxic drugs is often the cause of inefficient treatment of cancer with potent antitumor drugs. While many mechanisms of resistance occur, the mechanism of "multidrug resistance" (MDR) has received particular attention (ENDICOTT and LING, 1989). Most often, this type of resistance extends to several anticancer drugs of unrelated structural classes and mechanisms of action. A common feature of MDR is overexpression of a particular class of transmembrane glycoproteins called P-glycoproteins (Pgp) which serve as transport proteins rapidly effluxing antitumor drugs out of the tumor cells as soon as they have entered through the membrane. As a consequence, Pgp transporters decrease intracellular drug concentrations below their active threshold. Numerous *in vitro* studies have described agents which can restore the sensitivity of MDR tumor cells, including cyclosporin A at clinically achievable concentrations (TWENTYMAN, 1992). Moreover, this effect can be dissociated from immunosuppression, as non-immunosuppressive analogs have been shown to retain resistance modifier activity and some are even more potent than cyclosporin A. One such analog from Sandoz Pharma AG called SDZ PSC 833 is approximately tenfold more potent than Sandimmun® as a resistance modifier and is currently undergoing clinical trials (BOESCH et al., 1991). The structure of SDZ PSC 833 is shown in Fig. 3.

2.5 Anti-HIV Activity

A possible beneficial effect of Sandimmun® in HIV disease has been proposed as early as 1986 (ANDRIEU et al., 1986). The rationale is that activation of $CD4^+$ cells which is required for HIV replication (ZACK et al., 1990;

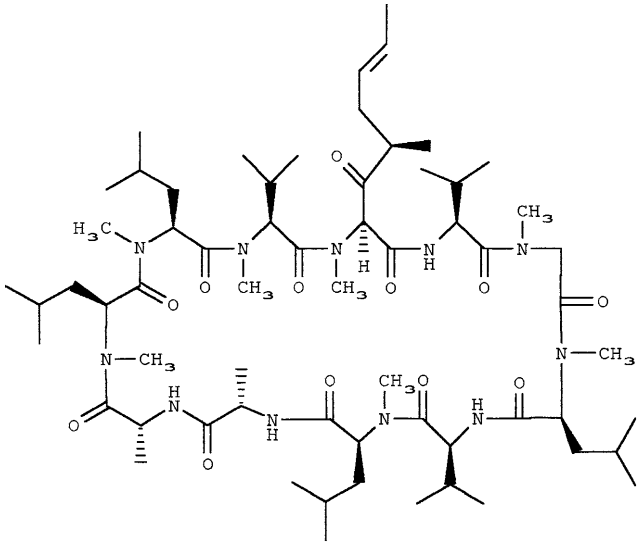

Fig. 3. Structure of SDZ PSC 833.

STEVENSON et al., 1990) is inhibited by CsA. In addition, CsA would inhibit the initiation of an autoimmune process involving killing of HIV infected lymphocytes by cytotoxic cells and may also counteract HIV-induced apoptotic cell death of $CD4^+$ cells (HABESHAW et al., 1990).

A thorough investigation of a series of immunosuppressive and non-immunosuppressive cyclosporins was performed in the preclinical research laboratories at the Sandoz Research Institute of Sandoz Pharma AG in Vienna, Austria. Compounds were evaluated for antiviral, cytotoxic, and for immunosuppressive activity *in vitro*. It was found that some non-immunosuppressive analogs of Sandimmun® were equal or even superior in their antiviral activity without being cytotoxic. One such analog, SDZ NIM 811 (Fig. 4), is comparable to Sandimmun® re-

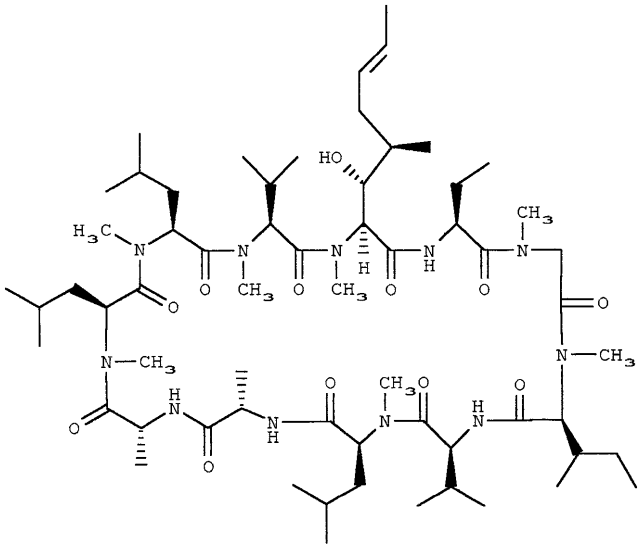

Fig. 4. Structure of SDZ NIM 811.

garding oral bioavailability and pharmacokinetics in animals and appears to be of lower nephrotoxicity (ROSENWIRTH et al., 1994).

3 Mode of Immunosuppressive Action

In addition to its clinical use as an immunosuppressant CsA has also been widely employed as an experimental tool for basic research. It has helped to understand the biochemical events needed to translate a signal from the T cell surface to the nucleus and the pathophysiological processes involving lymphocyte activation in a variety of diseases.

Early immunological studies revealed that CsA exerts specific effects on T cell lymphokine transcription (KRONKE et al., 1984). Because T cells are prominent in the cellular immune response, studies on the mechanism of immunosuppression by CsA have mainly focused on its role in regulating gene expression in T lymphocytes. To place CsA activity in perspective a summary on T cell activation is first given below.

3.1 Introduction to T Cell Activation

A schematic representation of the cellular immune response with emphasis on the central role of the activated T lymphocytes is shown in Fig. 5. For T cell activation, the antigen receptor on the T cell surface interacts with the processed antigen exposed in the proper histocompatibility context on the surface of the antigen presenting cell (APC; cf. Fig. 5). In the presence of additional accessory interactions between the T cell and the antigen presenting cell, antigen recognition leads to biochemical events which finally result in proliferation, differentiation, and maturation of the T cell to T effector cells with specific immunological function, e.g., helper and cytotoxic suppressor functions. The multiple steps involved in this process, namely signal recognition, signal transduction to the nucleus, resulting in gene activation, expression of growth factor receptors, growth factor synthesis and cell proliferation, are briefly reviewed (RYFFEL, 1989).

3.1.1 Signal Recognition

3.1.1.1 First Signal

After uptake and limited proteolysis, the antigen processed by the antigen presenting cell is recognized in the context of the major histocompatibility complex (MHC) by the antigen receptor on T lymphocytes (Fig. 5). The binding of antigen to the T cell receptor is an absolute requirement for T cell activation under physiological conditions. The T cell receptor is a multicomponent structure consisting of the clonotypic α and β (or γ and δ) chains and the invariant CD3 subunits γ, δ, , ζ, and η. The complete assembly of all components is required for cell surface expression, and thus for antigen receptor function. The ζ and η subunits of CD3 most likely transduce to the cytoplasm the activating signals originating from antigen recognition by the T cell receptor. Antibodies against the CD3 complex can induce T cell functional responses that are identical to antigen-induced responses, regardless of antigen specificity. In addition, the two transmembrane proteins CD4 and CD8 expressed on helper and cytotoxic T cells participate in the interaction between the T cell and the antigen presenting cell by binding to MHC class II and I molecules, respectively. Originally, they were called coreceptors because their association with an intracellular enzyme facilitates signaling during T cell activation (JANEWAY et al., 1989).

3.1.1.2 Costimulatory Signals

In addition to the molecular interactions between the T cell receptor, CD3, CD4, or CD8 and the antigen presenting cell, costimu-

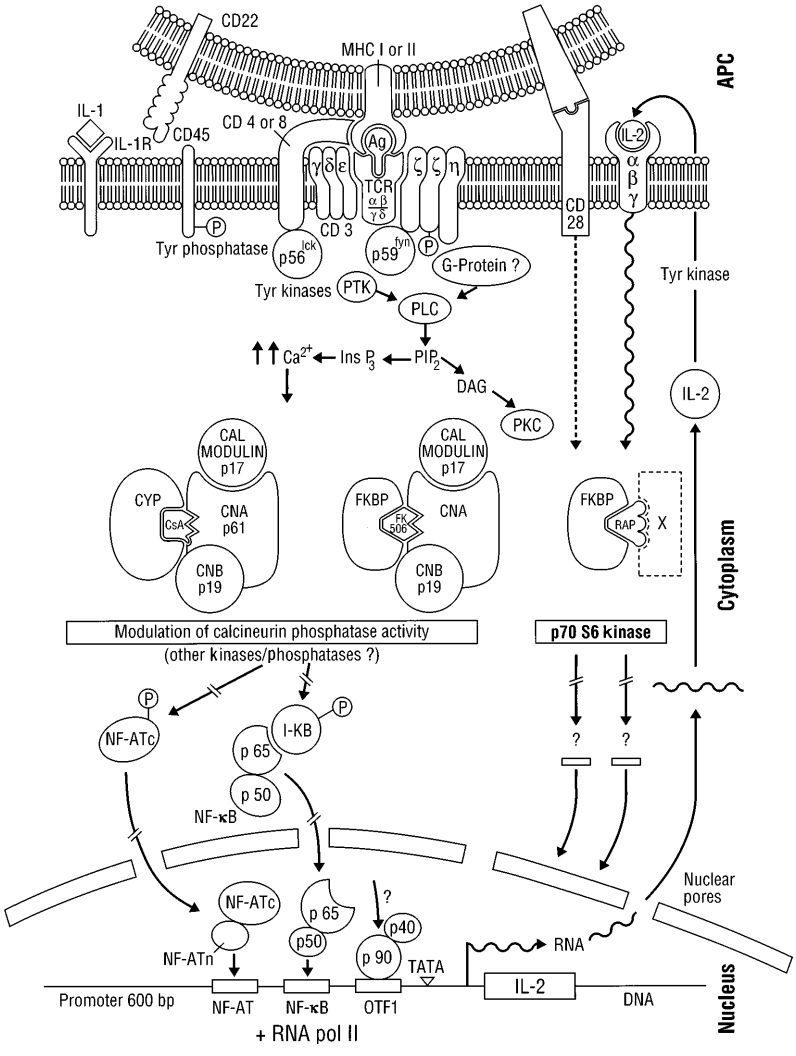

Fig. 5. CsA and FK506 both interfere, by binding to their respective immunophilins, with the function of intracellular molecules that transmit calcium-associated signals between the T-cell receptor (TCR) and the activation of lymphokine genes (IL-2) in the nucleus. Transcriptional regulation of IL-2 gene expression is modulated by the combination of transcription factors (e.g., NF-AT, NFκB, OTF-1) interacting with their corresponding recognition sites at the IL-2 promoter. These DNA/protein complexes, together with RNA polymerase II (RNA pol II), result in the antigen-inducible transcription of IL-2. Potential intervention sites for the pentameric complex (calcineurin A (p61), B (p19), calmodulin (p17), immunophilin, drug), involving, e.g., modification and translocation of antigen-inducible transcription factors (NF-AT; NFκB (p50, p65)), are indicated (II). CsA and FK506 interfere with the Go to G1 transition of the cell cycle, whereas rapamycin interferes with the G1 to S transition (for details, see text) (adapted from BAUMANN and BOREL, 1992).