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Research review paper

Cyclosporin A — A review on fermentative production, downstream processing and pharmacological applications

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ABSTRACT

In present times, the immunosuppressants have gained considerable importance in the world market. Cyclosporin A (CyA) is a cyclic undecapeptide with a variety of biological activities including immunosuppressive, anti-inflammatory, antifungal and antiparasitic properties. CyA is produced by various types of fermentation techniques using *Tolypocladium inflatum*. In the present review, we discuss the biosynthetic pathway, fermentative production, downstream processing and pharmacological activities of CyA.

Contents

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1. Introduction

Microorganisms are being used for thousands of years to supply fermented products such as bread, beer, wine, distilled spirits, vinegar, cheese, pickles and many other traditional regional products. The importance of microbes increased significantly during World War I during which development of fermentation, bioconversion, and enzymatic processes yielded many useful products such as amino acids, nucleotides, vitamins, organic acids, solvents, vaccines and polysaccharides. A major segment of these products are represented by secondary metabolites such as antibiotics. Many antibiotics have been used for purposes other than killing or inhibiting the growth of bacteria and/or fungi. These include hypocholesterolemic agents, immunosuppressants, anticancer agents, bioherbicides, bioinsecticides, coccidiostats, animal growth promoters, and ergot alkaloids (Demain, 2000).

Clinically, immunosuppression is defined as the inhibition of an immune response while avoiding the complications of immunodeficiency (Halloran, 1996). Patients who undergo solid organ transplantation require life-long immunosuppressive therapy to prevent allograft rejection. The success of post-transplantation patient care largely lies on the appropriate utilization of immunosuppressants. Immunosuppressants are a class of drugs which are capable of inhibiting the body's immune system. Many of the agents included in this category are also cytotoxic (cell poisons) and are used in the treatment of cancer. These drugs are used in organ transplant patients to prevent rejection of the organ by the body by decreasing the body's own natural defense to foreign bodies (such as the transplanted organs), and are also useful in the treatment of autoimmune diseases. The classification of immunosuppressants based on their primary sites of action is shown in Table 1.

Cyclosporins are a group of closely related cyclic undecapeptides produced as secondary metabolites by strains of fungi imperfecti, *Cylindrocarpum lucidum* Booth and *Tolypocladium inflatum* Gams isolated from soil samples (Dreyfuss et al., 1976; Borel et al., 1976). Both strains were isolated from soil samples collected in Wisconsin (USA) and Hardanger Vidda (Norway). CyA is the main component of this family of cyclic peptides containing 11 amino acids. CyA was isolated in the early 1970s on the basis of its ability to inhibit a mixed lymphocyte reaction (MLR), a measure of alloreactivity. CyA can be considered as the first of this kind of drug of a new generation of immunosuppressants. It is probably the first one to demonstrate the feasibility of an immunopharmacologic approach to the modulation of the immune response by drugs.

The introduction of CyA made an important advance in the immunotherapy of bone marrow and organ transplantations. CyA is one of the most commonly prescribed immunosuppressive drugs for the treatment of patients with organ transplantation and autoimmune diseases including AIDS owing to its superior T-cell specificity and low levels of myelotoxicity (Kahan, 1984; Schindler, 1985).

The organisms reported to produce CyA include *T. inflatum* (Agathos et al., 1986), *Fusarium solani* (Sawai et al., 1981), *Neocosmospora varinfecta* (Nakajima et al., 1988) and *Aspergillus terreus* (Sallam et al., 2003). CyA is reported to be produced by submerged culture fermentation (Agathos et al., 1986; Survase et al., 2009d), static fermentation (Balaraman and Mathew, 2006), solid state fermentation (Survase et al., 2009a), and also synthesized enzymically (Billich and Zocher, 1987).

Presently, CyA is available in the US market under brand names as Neoral®, Sandimmune®, Sandimmune® I.V by Novartis Pharmaceutical Corporation, USA; Gengraf® from Abbott Laboratories, USA; Restasis® from Allergan Inc USA; Apo-cyclosporin from Apotex Advancing Generics, Canada and Rhoxal-cyclosporin from Rhoxal-pharma, USA. In India, Panium Bioral® by Panacea Biotech Ltd., Arpimune® from RPG Life Sciences and CyclophilME® from Biocon India Ltd. are available in the market.

Immunosuppressants which have gained considerable importance in the world market include cyclosporin A (CyA), tacrolimus, rapamycin and mycophenolate mofetil. In the present review, we discuss the chemical structure, pharmacological activities, biosynthetic pathway, fermentative production, downstream processing, pharmacokinetics and toxicity of CyA.

2. History

In March 1970, in the Microbiology Department at Sandoz Ltd., Basel, a Swiss pharmaceutical company, a fungus *T. inflatum* Gams was isolated by B. Thiele from two soil samples, the first from Wisconsin, USA and second from the Hardanger Vidda in Norway. In 1973, CyA was purified from the fungal extract of *T. inflatum* and in 1975 complete structural analysis was established (Wenger, 1982). CyA was first investigated as an anti-fungal antibiotic (Dreyfuss et al., 1976), but Borel et al. (1976) discovered its immunosuppressive activity. This led to further investigations into its properties involving further immunological tests and investigations into its structure and synthesis. CyA was approved by the USFDA for clinical use in 1983 to prevent graft rejection in transplantation. It took 12 years for CyA to be developed into a drug Sandimmune® and was first registered in Switzerland. In 1984, synthetic CyA was produced. It was then possible for the CyA to be chemically modified in every possible way. However, none of the derivatives were found to have greater potency or lower side effects than CyA itself.

Before the introduction of CyA, the immunosuppressants used were methotrexate, azothioprine and corticosteroids. Beveridge (1986) reported that they block cellular division non-specifically and thereby inhibit the proliferation of the immunocompetent cells too which were attributed to their side effects. In contrast, CyA does not cause myelotoxicity and/or impaired the proliferation of hemopoietic stem cells (von Wartburg and Traber, 1986; Borel, 1981). Mcintosh and Thomson (1980) reported that CyA suppresses lymphocytic function without damaging the phagocytic activity and migratory capacity of the reticuloendothelial system which made its use in clinical transplantation attractive. The discovery of CyA led the way to an era of selective lymphocyte inhibition. It enabled expertise in clinical, technical and immunobiological aspects of transplantation to be put into practice and changed the face of transplantation.

3. Chemical structure

CyA is a neutral lipophilic cyclic polypeptide consisting of 11 amino acids and representative of this class which differs in their amino acid composition. It has molecular weight of 1202 and a molecular formula $C_{62}H_{111}N_{11}O_{12}$ (Fig. 1.). The acid hydrolysis of CyA showed that it is made up of eleven amino acids, ten of which are known aliphatic amino acids but the amino acid at position one was unknown (Wenger, 1982). All the amino acid residues have the 2*S* configuration, except for the alanine residue at position 8 which has the 2*R* configuration and achiral sarcosine at position 3. Amino acid residues