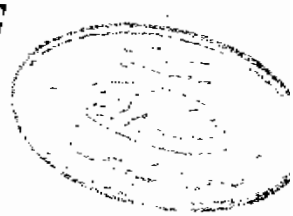
THERAPEUTIC DRUG MONITORING OF CYCLOSPORIN



Essay

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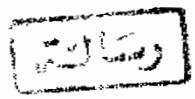

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For the sake of knowledge:

I have finished my study, and during the period of this task I had the houner to be supervised by great proferrors.

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On the occasion of ending this study. I am kindly asking **Dr. GIHAN KAMAL ALI,** Ass. Prof. of clinical pathology. Ain Shams University. and **Dr. OLA EL DEMERDASH,** lecturer of general surgery, Ain Shams University, to accept my deep appretiation for their helpful instructions which put on the right way.

Lastly, my cordial gartitude to my husband, mother. father, and friends for their continuous encouragement. help and patience.

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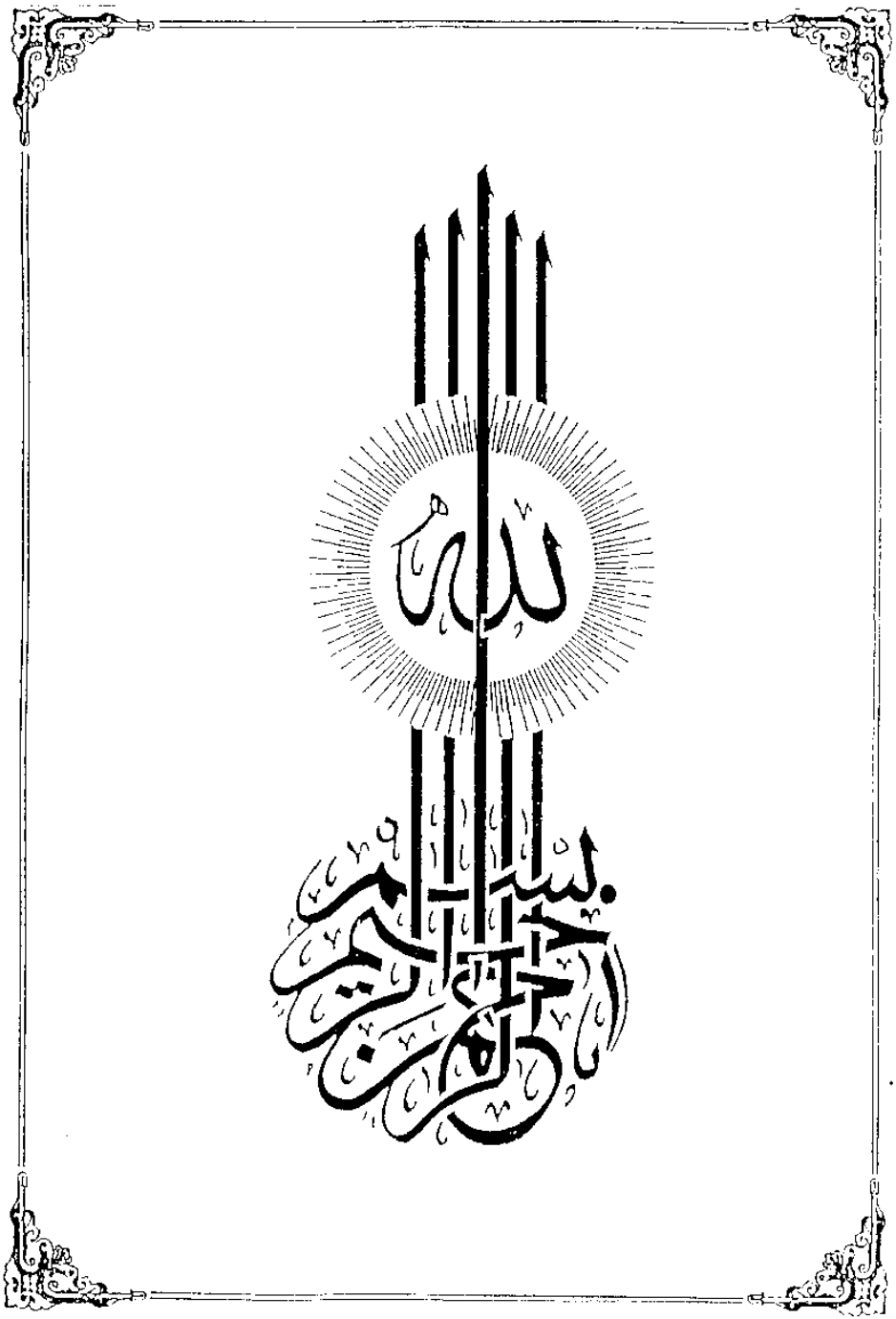

CONTENTS

INTRODUCTION

Cyclosporin is a unique immuno suppressive agent isolated from the fungus tolypocladium inflatum gams (NRRL 8044). It has been approved for use in generalorgan transplantation and for treatment of autoimmune disease (Kahan, 1984).

It has been successfully used for kidney, liver, pancreas, heart, lung and bone marrow transplants. Cyclosporin has been used as the sole immuno suppressant (Marion, 1984) and with prednisone (Najarian, 1983), it was hoped that cyclosporin would extend the degree of tissue mismatch acceptable for successful transplantations. In general, graft survival has been significantly improved.

Cyclosporin has a narrow therapeutic range and in order to avoid signs of cyclosporin toxicity especially nephrotoxicity which appears to be close related, concentrations of the drug must be monitored during therapy (Amadeo, 1989).

AIm of work:

The aim of the present study aims to give a detailed review of cyclosporin, its synthesis,

structure, mode of action, pharmacokinetics, clinical use & toxicity.

A special emphasis will be directed to laboratory techniques in a trial to sort out the advantages and disadvantages of each technique, encoutered fallacies and the possible ways of overcoming any source of variance which might then improve the performance characteristics of the chosen technique.

CYCLOSPORIN

A. Historical BackGround, Synthesis and Structure:

Cyclosporin is first isolated from two strains of fungi imperfecti from soil samples by the microbiology department at Sandoz* in Bastle (Cylindrocarpon lucidum and trichoderma polysporum). The latter from which cyclosporin is now produced being more correctly known as Tolypocladium inflatum (Gams) as an antifungal agent of limited activity (Borel, et al.. 1977).

Clinical trials of the drug in renal transplantation began in Cambridge in 1978, (Calne, 1981), and over the last years considerable experience with the use of cyclosporin has been obtained in a number of centers.

Cyclosporin is an exciting new immunosuppressive drug, which has proven to be a potent agent in a wide variety of experimental models of tissue transplantation, and promise to be a valuable addition

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* Sandoz: East Hanover, NJ.

to the immunosuppressive armamentarium in clinical organ transplantation (Borel, 1981).

Recently the drug has been synthesized which should allow many questions concerning its mechanism of action and toxicity to be explored precisely and also allow other molecules with similar immunosuppressive properties but less toxicity to be synthesized. (Aeder, et al., 1983).

Cyclosporin has been shown effective in suppressing host versus graft rejection in heterotopic organ transplants and is approved for use in renal, cardiac, hepatic and pancreatic organ transplants, its use in bone marrow transplant and suppression of autoimmune disease is under investigation, but appears to be promising (Faynor, 1984).

The drug is a hydrophobic, neutral, cyclic peptide composed of 11 amino acids. It has a molecular weight of 1203. most of its comprising amino acids are hydrophobic, thus cyclosporin is only soluble in lipids or organic solvents, (Faynor, 1984).

B. Mechanism of Action:

Cyclosporin has been shown to inhibit the proliferative response of lymphocytes to concanavalin

A, phytoheamagglutinin and pokeweed mitogen in vitro (Borel, 1981). If cyclosporin is added 48 hours after the addition of mitogen to a culture, then there is no inhibition of proliferation and the effect is reversed by washing the lymphocytes and reexposing them to mitogen. Complete inhibition of the mixed lymphocyte reaction (MLR) by cyclosporin has been demonstrated in several species including man, (Hess and Tustchka, 1980). The generation of cytotoxic lymphocytes in the MLR is prevented by cyclosporin but once generated, then cyclosporin has no effect on their cytotoxic activity. therefore theoretically cyclosporin would not be expected to inhibit the secondary MLR response nor the generation of cytotoxic T lymphocytes in sensitized animals (Hess and Tutschka, 1980).

There is a growing amount of evidence that the action of cyclosporin is directed predominantly against T helper lymphocytes. This effect on the T helper cell appears now to prevent the production of lymphokines especially Interleukin 2 (T cell growth factor) Borel (1981). In this way the generation of the cytotoxic T-cells from the cytotoxic T-cell precursors is prevented.

Cyclosporin may also have inhibited the maturation of cytotoxic T-cell precursor either by

preventing the development of receptors for Interleukin 2 (Hess, 1983) or by the inhibition of another lymphokine produced by the T helper cell which acts on the cytotoxic precursor cell and induces the production of Interleukin 2 receptors. Interleukine 1 production by the macrophage as well as Interleukine 3 production (Colony stimulating factor) are also propably inhibited by cyclosporin (Lafferty, 1983).

Although in general cyclosporin has not been thought to inhibit the function of B lymphocytes, (Borel, 1977), there is now some evidence to the contrary both in man and mouse (Bower and Hinrichs, 1983). Klaws and his colleagues (1980) have demonstrated a cyclosporin sensitive subpopulation of T-independant B lymphocytes in the mouse.

No evidence for a specific cyclosporin receptor has been found, nor any differential binding of cyclosporin to B cells, helper T-cells or suppressor T-cells (Lafferty, 1983).

Thus although the action of cyclosporin remains to be completely elucidated, it is becoming clearer that its predominant effect is directed at T lymphocytes at an early stage of the induction of the

immune response to an antigen. Much of this activity is related to the inhibition of the production of lymphokines, such as Interleukin 2, by the T helper cell thus preventing the Interleukin 2 induced proliferation of cytotoxic T-cell precursors.

C. Clinical Indications:

Cyclosporin is a unique immunosuppressive agent. It has been approved by the Food Drug Association (FDA) for use in organ transplantation. It has been successfully used for kidney, liver, pancreas, heart, lung and bone marrow transplants. It has been used both as the sole immunosuppressant and with prednisone or prednisone and azathioprine (Najarian, 1983).

In general graft survival has been signifcantly improved and in some cases steroids have been completely avoided. Thus cyclosporin, with or without steroids, has made a most impressive debut in renal transplantation in term of patient and graft survival (Bowers L.D., 1989).

Cyclosporin is also one of the most exiting new therapeutic finds in dermatology. It is exciting because its effects are profound and unexpected in treatment of many diseases as psoriasis and dermatitis

(Baker B.S., 1984).

The drug has been also shown to be applicable in occular inflamatory disorders including: disorders of the occular surface, transplantation, and intraoccular inflamatory diseases (Foster C.S., 1984).

It has been also used therapeutically in immunopharmacological treatment in mythenia gravis, as immunosuppressive therapy in rheumatological diseases and in primary glomerulonephritis to treat patients with idiopathic nephrotic syndrome (Lancet, 1986).

There are also cyclosporin trials in diabetes mellitus and its effects of immunosuppression in insulin dependant diabetes mellitus (IDDM), (Canadian Europian Study Group, 1987).

D. Pharmacokinetics:

1- Absorption:

Cyclosporin is absorbed from the upper small bowel Kahan, (1983) reported that a variable fraction of the oral dose of cyclosporin was absorbed, ranging from 4% to 26%. In 1984, he further reported that in most patients, bioavailability increased with continued therapy from a mean pretransplant value of 57% after two weeks of treatment. Similar

bioavailability data were reported by Wood, (1983), in his study, absolute bioavailability of the oral solution at steady stage ranged from 20% to 50% (mean 34%) Peak concentrations of cyclosporin in blood and plasma occur two to four hours after administration.

Factors affecting absorption:

A number of reports describe several different factors that appear to affect the absorption of orally adiminstrated cyclosporin.

I- With most patients, as therapy continues, bioavailability increases overtime (Kahan, 1983).

II- Paediatric patients undergoing renal, heart or liver transplants require and tolerate higher doses (per kilo gram of body weight) than those used in adults (Hoyer P Fand Offner G, 1984).

III- Orthotopic liver transplant patients with diarrhea, liver failure and external bile damage (Ptachcinski R J, 1985), as well as paients with stable liver disease with cholestasis, (Vankataramanan, 1985), demonstrate malabsorption.

IV- The results of studies exploring the effect of food on absorption are conflicting, therefore definitive conclusions cannot be drawn (Keown P.A., et al., 1982).

2- Distribution:

Cyclosporin is widely distributed throughout body tissues. The volume of distribution has been reported to vary from an average of 3.5 L/Kg to 13 L/Kg, of body weight (Beveridge, 1982).

The liver, pancreas and fat contain higher concentrations of cyclosporin than are found in plasma, indicating preferential uptake in these tissues, (Ptachcinski RJ, 1985).

Fifty per cent to 70% of the cyclosporin dose found in whole blood is bound to cellular fraction. Of this fraction, erythrocytes are the primary binding component (80%), while lymphocytes bind to approximately 4% to 9% of the Sandimmune dose (Beveridge T., 1982).

The remaining 30% to 50% found in the plasama fraction is almost all bound to plasma proteins (85% to 90%), primarily lipoproteins, (Sgoutas D., et al., 1986).

It appears that binding to plasma proteins and erythrocytes is linear, whereas leucocytes appear to bind the drug in a non linear fashion (Lemaire, M., 1982).

3- Cyclosporin metabolism and metabolites:

Cyclosporin undergoes extensive metabolism (99%) (Beveridge, T., 1982). The parent drug is metabolised to approximately 17 metabolites, 13 of which have been isolated and identified (Maurer, G., 1985).

The cytochrome P-450 liver microsomal enzyme system is most likely the metabolic pathway by which cyclosporin undergoes biotransformation (Robinson, 1983).

All identified metabolites retain their cyclic undecapeptide structure and are more hydrophilic than the original compound. Like the parent compound they are almost insoluble in water or in saturated aliphatic hydrocarbons (Maurer, et al., 1984).

Identified metabolites contain the intact cyclic oligopeptide structure of the parent drug but structure modifications consist of mono and dihydroxylation and N-demethylation at various sites on the cyclosporin molecule (Maurer, G., 1985).

In vitro immunosuppressive activity of four metabolites (Met 1,Met 8, Met 17 & Met 21) has been compared with that of cyclosporin for inhibition of