REVIEW ARTICLE

Immunophilins: Receptor Protein For Immunosuppressant Drugs

Neelam Vashist, R. Agarwal, R. Sharma, J.Singh*, S.K. Sharma, D.P. Pathak**

Introduction

Immunophilins are natural intracellular receptor proteins that interact with proteins to guide their proper folding and assembly (1). These receptor proteins bind with high affinity to immunosupperessant agents such as cyclophilin for cyclosporine- A and FK-506 binding protein (FKBP) for FK-506 and rapamycin. Most effective immunosuppressive drugs in routine use are the calcineurin inhibitors, cyclosporine and tacrolimus, which target intracellular signaling pathways induced, as a consequence of T cell receptor activation. Although, they structurally differ and bind to distinct targets but they inhibit T cell signal transduction usually by the same mechanism2. Cyclosporine and tacrolimus do not act per se as immunosuppressive drugs but these drugs bind to an immunophilin (cyclophilin for cyclosporine or FKBP-12 for tacrolimus), resulting in subsequent interaction with calcineurin to block its phosphatase activity (2). Calcineurin catalyzed dephosphorylation is required for movement of a component of the nuclear factor of activated T lymphocytes (NFAT) in to the nucleus. NFAT, in turn is required to induced a number of cytokine genes, including that for interleukin-2, a prototypic T cell growth and differentiation factor. Some immunophilins are HSP90 binding co chaperones that affect steroid receptor function ie regulates glucocorticoid response to stress related disorders (3). Immunophilins have been implicated in several cardiovascular disorders including vascular stenosis, atherosclerosis, heart failure and arrhythmias (4). The 52 kDa FK-506 binding protein (FKBP52) has been shown to be an important regulator of androgen receptor in cellular and whole animal models and represents an attractive target for the treatment of prostate cancer (5).

Cyclosporine- A

Cyclosporine-A, is a cyclic peptide of 11 amino acid is produced by the fungus beauveria nivea. It preferencially inhibits antigen triggered signal transduction in T lymphocytes, blunting expression of many lymphokines including IL-2 and expression of antiapoptotic proteins. Cyclosporine forms a complex with cyclophilin. This complex binds to calcineurin including Ca2+ stimulated dephosphorylation of the cytosolic component of NFAT. Calcineurin phosphatase activity is inhibited after physical interaction with cyclosporine/cyclophilin complex. This prevents NFAT dephosphorylation such that NFAT does not enter the nucleus, gene transcription is not activated and T lymphocytes fail to respond to specific antigen stimulation (2). Cyclosporine also increases expression of transforming growth factor beta (TGF-beta). Cyclosporin -A can be given orally or i.v. Gelatin capsules and solutions are available for oral use. Gelatin capsules absorbed slowly with 20-50% bioavailability. Thus modified microemulsion formulations have become most widely used preparations. It is extensively distributed outside the vascular compartment. It is metabolized in the liver by cytochrome CYP 3A and to a lesser extent in gastrointestinal tract and kidney. At least 25 metabolites have been identified in human bile, feces, blood and urine. Elimination is mainly through bile in to the faeces and about 6% being excreted in urine (2).

Clinical indications for cyclosporine are kidney, liver, heart and other organ transplantation. Cyclosporine usually is combined with glucocorticoids and either azathioprine or mycophenolate and most recently, sirolinus (6). Dose of cyclosporine varies depending on organ transplanted and the other drug used in combination. Closed monitoring

From the Maharaja Surajmal Institute of Pharmacy affiliated to Guru Gobind Singh Indraprastha University, C-4, Janakpuri, New Delhi, *PGIMS, Rohtak & **DIPSAR, Pushp Vihar, New Delhi-110017- India

Correspondence to : Dr Neelam Vashist, Assistant Prof., Maharaja Surajmal Institute of Pharmacy, C-4 Janakpuri, New Delhi - 110058

of blood levels of drug is essential. It is used in severe cases of rheumatoid arthritis that have not responded to methotrexate. Cyclosporine reportedly is effective in psoriasis, Behcet's acute ocular syndrome, endogenous uveitis, inflammatory bowel disease and nephritic syndrome, even when standard therapies have failed. It is also known to suppress prostate cancer cell growth. It is being considered as a potential treatment for some cases of asthma (2). Cyclosporine has also been used as an off label drug for treatment of various inflammatory skin diseases including atopic dermatitis, blistering disorders &connective tissue disease (7). Principal adverse effects of cyclosporine therapy are nephrotoxicity, hypertension, tremor, hirsutism, hyperlipidemia and gum hyperplasia (1, 2).

ISATX 247- It is a new oral semi-synthetic structural analogue of cyclosporine. Cyclosporine molecule is modified at the first amino acid residue. It is more potent than cyclosporine as calcineurin inhibitor. The drug is in clinical development as a immunosuppressant agent. Phase II clinical trials show less nephrotoxicity with less frequent glucose intolerance compared to tacrolimus treated patients (2).

Tacrolimus

Tacrolimus (FK 506) is a macrolide, produced by Streptomyces tsukubaensis (2). Tacrolimus inhibits cellular immune response and humoral immune response but to a lesser extent. Like cyclosporin, tacrolimus inhibits T-Cell activation by inhibiting calcineurin. Tacrolimus binds to an intracellular protein, FK506-binding protein-12 (FKBP-12). A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin then forms and calcineurin phosphatase activity is inhibited. The inhibition of phosphatase activity prevents the dephosphorylation and nuclear translocation of NFAT and leads to inhibition of T-Cell activation (2). Tacrolimus is available for oral use as capsule and solution for injection.

Standard Tacrolimus 5mg/day, single dose was increased to 6mg/day at steady state. At steady state in healthy volunteers, administered tacrolimus single dose 4 mg/day, the median time to Cmax (tmax) was 2 hrs. Gastrointestinal absorbtion is incomplete and variable. Presence of food reduces the rate and extent of absorption of tacrolimus. Tacrolimus is strongly bound to erythrocytes and distribution between blood and plasma varies widely. Protein binding in plasma mostly to alpha1-acidglycoprotein albumin is upto 99%. Tacrolimus is widely distributed with a volume of distribution 1.4L/kg and 0.85L/kg in renal and liver transplant recipients receiving i.v. tacrolimus. Tacrolimus also crosses the placenta and is distributed in the breast milk. It undergoes metabolism by cytochrome P450 CYP3A4 isoenzyme in liver. The t ½ of Tacrolimus as about 12 hours. Less than 0.5% of the unchanged drug is excreted in faeces or urine. Elimination of tacrolimus metabolites is predominantly via biliary route (>95%).

Tacrolimus is approved for the prophylaxis of solid-organ allograft rejection in a manner similar to cyclosporine and is used off label as rescue therapy in patients with rejection episodes despite therapeutic levels of cyclosporine (2). In 2008, the majority of liver, kidney, heart, lung, intestine, heart, lung & kidney-pancreas transplant recipients reported to US Procurement& Transplantation Network& the Scientific Registry of Transplant recipients prescribed this agent at hospital discharge (8). Recommended initial oral doses are0.2mg/kg/day for adult kidney transplant patients, 0.1-0.15 mg/kg/day for liver transplant patients, 0. 075 mg/kg/day for heart transplant patients and 0.15- 2.0 mg/kg/day for pediatric liver transplant patient .It also suppress prostate cancer growth, improves functional recovery of damaged sciatic nerves. Recently it has also been used to treat segmental vitiligo in children and for atopic dermatitis and psoriasis (1). Barraclough *et al* (9) reported that single daily dose of Tacrolimus for prevention organ rejection was equally effective as compared to twice daily dose.

Nephrotoxicity and neurotoxicity (tremor, seizures, headache, and motor disturbances), gastrointestinal complaints, hypertension, hyperkalemia, hyper glycemia and diabetes are associated with Tacrolimus use. Increased risk of secondary tumors & opportunistic infections may occur. Tacrolimus does not adversely affect uric acid or LDL cholesterol (2).

Sirolimus

Sirolimus, is a macrocyclic lactone derived from Streptomyces hygroscopicus. Sirolimus inhibits T lymphocyte activation and proliferation downstream of IL-2 and T cell growth factor receptors. Sirolimus binds to FKBP-12. However, the sirolimus-FKBP-12 complex does not affect calcineurin activity. It binds to and inhibits

a protein kinase, called m TOR, which is a key enzyme in cell cycle progression. Inhibition of mTOR, blocks cell cycle progression at G 1- -S phase transition.

Sirolimus is absorbed rapidly when given orally and reaches a peak blood concentration (C max) within 1-2 hr. after administration. Systemic availability is about 15%. A high fat diet decreases Cmax by 34% and about 40% sirolimus bound to plasma proteins. It is metabolized by CYP3A4 and is transported by P glycoprotein. The t ½ after multiple doses in stable renal transplant patient is 62 hrs. Ninety percent of the parent compound is excreted in faeces with a very little urinary excretion.

Sirolimus is indicated for prophylaxis of organ transplant rejection (kidney, liver, pancreas, heart) usually in combination with a reduced dose of calcineurin inhibitor and glucocorticoids. In patients experiencing high risk for calcineurin inhibitor induced nephrotoxicity, sirolimus has been used with glucocorticoids or mycophenolate to avoid permanent renal damage. Sirolimus blood levels generally targeted between5-15 ng/ml. daily maintenance dose should be reduced about 1/3rd in patients with hepatic impairment. A newer indication for sirolimus is avoidance of calcineurin inhibitors even when patients are stable to protect kidney function (10). Sirolimus has been incorporated into stents to reduce coronary restenosis and blood vessel occlusion (2).

Use of sirolimus in renal transplant patients is associated with increase in serum cholesterol and triglycerides. Sirolimus per se is not nephrotoxic, patients treated with sirolimus plus cyclosporine has impaired renal function. Other adverse effects include anemia, leucopenia, thrombocytopenia, mouth ulcers, hypokalemia, proteinuria, delayed wound healing and gastrointestinal side effects (2).

Everolimus

Everolimus (40-o-[2-hydroxy]ethylrapamycin) is a proliferative signal inhibitor that may be of benefit in decreasing rejection in cardiac transplantation. It is closely related chemically and clinically to Sirolimus but has distinct pharmacokinetics. The main difference is a shorter half life and thus a shorter time to achieve steady state concentration of the drug. (Dosage on a milligram/kg basis is similar to sirolimus). Thompson *et al* (11) reported that everolimus was safe and effective treatment option for patients with low to intermediate grade unresectable or metastatic pancreatic neuroendocrine tumor that have progression on prior therapy.

As with Sirolimus, the combination of a calcineurin inhibitor and an mTOR inhibitor produces worse renal function than does calcineurin inhibitor therapy alone, suggesting a drug interaction between mTOR inhibitors and the calcineurin inhibitors to enhance toxicity and to reduce rejection. The toxicity of Everolimus is similar to the toxicity profile of Sirolimus (2).

Tersirolimus

Temsirolimus is an ester of Sirolimus, selectively inhibits the mammalian target of rapamycin (m TOR) kinase with consequently blocking the translation of cell cycle regulatory proteins. It selectively binds to the immunophilin FK 506 binding protein 12kDa isoform (FKBP12) to form a complex with mTOR. When mTOR is bound with this complex, it is unable to phosphorylate protein translation factors namely the eukaryotic initiation factor 4 E binding protein I & S 6 kinase, which are downstream of mTOR in the phophatidylinositol 3-kinase/Akt/mTOR pathway. Thus, the translation of several key proteins regulating the cell cycle is inhibited and the cell cycle is blocked at the G1 phase.Temsirolimus is well established first line drug in post transplant immunosuppression. More recently, mTOR inhibitors have found important application for treatment of renal and hepatocellular cancer & mantle cell lymphomas (2). The FDA has approved temsirolimus for treatment of renal cancer. It prolongs survival and delays disease progression in patients with advanced and poor and intermediate risk renal cancer as compared to standard interferon- alpha treatment. For renal cancer temsirolimus is given in weekly doses of 25 mg, i.v . Poor bioavailability hampers the oral route of administration as <20% of drug reaches the plasma. It is metabolized by Cyp3A4. Temsirolimus has a plasma t1/2 of 30 hrs.. Its primary metabolite, sirolimus has a longer t1/2 of 53 hrs.Excretion of temsirolimus was primarily via faeces (78%) and renal excretion accounting for 46% of the administered dose. Hoy &Mc Keage (12) reported that therapy with i.v. temsirolimus 175 mg once weekly for 3 weeks followed by 75mg once weekly was significantly more effective than single agent chemotherapy in patients with relapsed and /or refractory Mantle cell lymphoma in phase III study.

Pimecrolimus

Pimecrolimus (Ascomycin) also called Immunomycin, FR-900520, FK520, is an ethyl analog of tacrolimus with strong immunosuppressant properties, produced by the fermentation of Streptomyces hygroscopicus. Pimecrolimus was approved by the FDA for treatment of dermatitis in patients>2 years of age(13). Its mechanism of action and side effects are similar to those of Tacrolimus. Burning is less common with pimecrolimus than with Tacrolimus. Pimecrolimus has less systemic absorption. Due to potensial for malignancy production, topical calcineurin inhibitors are not considered first line therapy in childhood atopic dermatitis(14). Tacrolimus and pimecrolimus should only be used as second line agents for short term& intermittent treatment of atopic dermatitis (eczema) in patients unresponsive to or intolerant of other treatments (2). It can be used to treat autoimmune diseases and can help in preventing rejection of organ transplant. Mionoona *et al* (15) reported long term beneficial effects after intralesion injection of 1% pimecrolimus in patients with orofacial granulomatosis.

Conclusion

Immunophilins are indicated in the treatment if certain autoimmune disorders including rheumatoid arthritis, asthma, psoriasis, atopic dermatitis, Crohn's disease and to prevent rejection of transplant organs. They are potential candidates for future studies in treatment of cardiovascular disorders, erectile dysfunction, prostate carcinoma and as neuroprotective agents in neuronal injuries.

References

1. Katzung B G, Masters S B, Trevor A J. In: Basic and clinical Pharmacology, 12th edition, Mc Graw Hill Lange, Toronto, 2012 .pp. 644.
2. Brunton L L, Chabner BA, Knollman BC. In: Goodman & Gilman's The pharmacological basis of therapeutics, 12th edition, Mc Graw Hill, New York, Toronto. 2011.pp. 1008-29.
3. Galigniana NM, Ballmer LT, Toneatto J, MLagadari J E, Galiniana MD. Regulation of the glucocorticoid responce to stress related disorders by the HSP90- binding immunophilin FKBP51. *J Neurochem* 2012; 122: 4-18.
4. Calderon-Sanchez E, Rodriguez-Hoyano M, Smani T. Immunophilins and cardiovascular complications. *Curr Med Chem* 2011; 18(35):5408-13.
5. De Leon J T , Nai G , Feauc-Garcia Y, Balsiger HA, Storer CL. Targeting the regulation of a receptor signalling by the heat shock protein 90 cochaperone FKBP52 in prostate cancer cells. *Proc Natl Acad Sci USA* 2011; 108 (29): 11878-83.
6. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. In : Harrison's Principles of Internal Medicine, 18 th edition, Mc Graw -Hill, New York, Tronto, Vol. II, 2012.pp.1907-2024.
7. Dehesa L , Abuchav A, Nuno-Gouzalez A, Vitiello M , Kerdel FA . The use of cyclosporine in dermatology. *J Drugs Dermatol* 2012; 11(8): 978- 87.
8. US Organ Procurement & Transplantation Network and the Scientific Registry of Transplant Recipients (online) Available from URL: http:// www us transplant. Org./ annual _ reports/ current [Accessed 2010, Dec 20]
9. Barrclough KA, Isbel NM, Johnson DW, Campbell SB, Staatz CE. Once versus twice daily tacrolimus: Are the formulations truely equivalent? *Drugs* 2011; 71(12): 1561-77.
10. Flechner SM, Kobashigawa J , Klintmalm G . Calcineurin inhibitor sparing regimens in solid organ transplantation: Focus on improving renal function and nephrotoxicity. *Clin Transplant* 2008; 22: 1-15.
11. Thompson LA, Kim M, Wenger SD, O'Bryant CL. Everolimus : A new treatment option for advanced pancreatic neuroendocrine tumor. *Ann Pharmacother* 2012 (E Pub ahead of print)
12. Hoy SM, Mc Keage K. Temsirolimus: in relapsed and /or refractory Mentle cell lymphoma. *Drugs* 2010; 70(14):1819-29.
13. Afshar M , Kotol P , Miller J , Gallo R, Hata T . The effect of pimecrolimus on innate immunity in atopic dermatitis subjects: a double mblind randomised vehicle controlled study. *Br J Dermatol* 2012; 10: 1111/j 1365-2133.
14. Solassol J, Mange a, Maudelonde T. FKBP family protein as promising new biomarkers for cancer. *Curr Opin Pharmacol* 2011; 11(4): 320-25.
15. Mionoona MD, Pollio A , Leuci S , Ruoppo E, Fortona G .Clinical behaviour and long term therapeutic response in orofacial granulomatosis patients treated with intralesional triamcinolone acetonide injaction alone or in combination with topical pimecrolimus 1%. *J Oral Pathol Med* 2012; 10; 1111/j 1600-0714.