Comparison of Cyclosporin A and Tacrolimus in the Field of Organ Transplantation

Ruixuan Wen
University of California, San Diego, San Diego, California, 92093, USA
rwen@ucsd.edu

Abstract: Organ rejection occurs when a patient’s immune system recognizes transplanted organ as foreign, initiating immune responses that ultimately destroys the transplant. Since organ transplantation is offered only after all the other treatments have failed, the rejection is hence fatal and requires immediate medical treatment. As a solution, immunosuppressive drugs are widely used to treat organ rejection. Calcineurin inhibitors (CNIs), a kind of non-depleting agents including but not limited to Cyclosporin A (CsA) and Tacrolimus (FK506), prevent T-cell activity intracellularly through inhibiting cytokine expression and T-cell proliferation. By comparing the structure, mechanism and application of Cyclosporin A and Tacrolimus, not only organ rejection can be better understood, the two immunosuppressant can be better evaluated and studied.

1. Introduction

1.1 Transplant failure devastates a patient’s life.
7–12 in every 100 recipients undergo transplant failure. Research conducted by Gill and Lowes on “the kidney transplant failure experience” recorded a female who received kidney transplant from her mate and encountered a transplant failure. Despite the fact that her renal function deteriorated as I said was and the transplant was no longer viable, the woman developed a series of adverse emotions (anger, disappointment, grief, loss and depression) —— she almost committed suicide. This is only a normal scenario of patients who encounter transplant failure [1].

1.2 Recipients’ immune systems detect the transplant as a foreign and carry out organ rejection.
According to organdonor.gov, the largest data platform of organ transplantation that each day, there are about 80 people receive organ transplants [2]. The transplanted organs are identified by patients’ Human Leukocyte Antigen (HLA) protein as a “non-self”, triggering an immune response that initiate the production of antigen-specific antibodies. Since every individual has his/her own sets of HLA genes, the degree of similarity between recipient and donor’s HLA genes, known as histocompatibility, determines the severeness of organ rejection——the more genetically compatible the recipient and the donor, the more endurable the recipient’s immune system is against the transplant [3].

As soon as recipient’s HLA protein detects the transplant, the recipients’ T-cells start to recognise alloantigens on the surface of a transplant through direct, semi-direct and indirect pathway of alloreognition. Once the T-cell receptor binds to MHC molecules on antigen-presenting cells[4], T-cells in host’s body are activated, generating large amounts of IL-2 and proliferative cytokines that promote and initiate the differentiation and clonal expansion of activated T-cells[5]——the initial process of T-cell activation is calcium dependent[4]. All the adaptive immune activities eventually lead
to attacks toward the transplant—if immediate treatments are not taken, the transplantation will culminate in failure.

1.3 Cyclosporin A and Tacrolimus are CNIs-based immunosuppressants.

Aiming at immune activity inhibition, Cyclosporin A (CsA) is an insoluble lipophilic cyclic undeca peptide isolated from a fungus called Hypocladium inflatum gams [6] that was found in a soil sample bought back by Sandoz employees on a business trip to Hardangervidda during the antibiotic screening programme (1958) [7]. The wide use of Cyclosporin A for preventing organ rejection is mainly attribute to its ability to prevent organ rejection through inhibiting T-cell activity. At the same time, the drug, compared to other immunosuppressants, is nontoxic for bone marrow stem cells and leads to fewer bacterial infections [8].

Similar to Cyclosporin A (CsA), tacrolimus is also an immunosuppressant belonging to Calcineurin Inhibitors (CNIs) group. The discovery of Tacrolimus was carried out by an Exploratory Research Laboratory in Japan in 1987. Isolating from a soil fungus, Streptomyces tsukubaensis, this 23-membered macrolide lactone (C_{44}H_{69}NO_{12}) has great therapeutic value in solid organ transplantation, especially for preventing rejection in pancreas, bone marrow, lung, kidney and heart [7].

2. Structural comparisons between Cyclosporin A (CsA) and Tacrolimus (FK506)

While both CsA and FK506 are cyclic, different bonds and synthetical strategies were made to form two structures. (see fig. 1)

Cyclosporin A (CsA), a polypeptide containing “7-methylated peptide bonds and three non-proteinogenic amino acids, a Bmt, L-2-aminobutyric acid (Abu) and D-alanine [9], is biologically synthesised by trans action of 3 enzymes in a process called NRPS (Non-Ribosomal Peptide Synthesis). While proteinogenic amino acids are derived from classical amino acid biosynthetic pathway, Bmt is biosynthesised by polyketide synthesis (PKS); meanwhile, D-alanine is directly supplied to start the Cyclosporin A synthesis as CySyn enzyme product does not carry out epimerase function. Due to the fact that the amino acid residues’ side chains are mainly composed by hydrophobic alkyl groups (e.g. Valine), Cyclosporin A (CsA) does not easily dissolve in water; therefore, the drug can easily penetrate into the fatty acid-based cell membrane.

In comparison, Tacrolimus (FK506) is a 23-member lactone biologically synthesised by a hybrid of PKS-NRPS (polyketide synthesis-non-ribosomal peptide synthesis) system. A module played role in the biosynthesis of FK506 generally consists of KS (ketoacylsynthase), AT (acyltransferase), and ACP for chain elongation. Optionally, each module may include DH (dehydratase), ER and KR. After the polyketide was formed, Lysine is added by FkbP enzyme product to cyclise the polyketide and form the final product —— Tacrolimus (FK506) [10].

![Figure 1. Structural Comparison between CsA and FK506](image)
3. Mechanism comparisons between Cyclosporin A(CsA) and Tacrolimus (FK506) immunosuppressive effect

3.1 T-cell activation is directly related to the mechanisms of Cyclosporin and Tacrolimus.
TCR-CD3 complex binds to MHC class II molecules on an antigen, leading to cytokine gene expression and T-cell activation. Meanwhile, the calcium-based system facilitates calcineurin’s function to remove a phosphate group from NFATp, leaving out NFAT (nuclear factor of activated T-cell) that initiate IL-2 gene transcription. IL-2 on the surface of a T-cell will eventually enable T-cell proliferation.

3.2 CsA and FK506 bind to immunophilins to form complex that inhibit T-cell proliferation, and FK506 solely interrupt the post-transcription of IL-2.
CsA and FK506 bind to immunophilins, a highly conserved group of proteins that possess binding ability to immunosuppressant[11]. Respectively, CsA binds to cyclophilin—a cytosolic protein that possesses peptidyl-proline-cis-trans isomerase (PPIase) activity—and FK506 binds to FKBP(Interestingly, one of the enzyme product that is involved in the PKS of FK506 is called FkbP)[10]. The CsA-cyclophilin complex or FK506-FKBP complex binds to calcineurin, a calcium dependent phosphatase, inhibiting the nuclear translocation and activation of NFAT(Nuclear factor of activated T-cell). Hence, the IL-2 transcription is stopped, leading to the inhibition of T-cell proliferation[4].

In addition to two drugs’ shared calcineurin/NFAT pathway, FK506 acts post-transcriptionally to inhibit cytokine expression, the factor binding to FK506 is undetermined.

3.3 CsA and FK506 affect JNK and p38 signalling pathway to block the transcription of NF-AT cis-element.
Transcription of IL-2 genes requires several transcriptional factors to functions; these transcriptional factors include AP-1, NF-kB, and NFAT. It is discovered that CNIs(including FK506 and CsA) not only have effects on calcineurin’s phosphatase activity, they also affect the activity of NF-kB and AP-1, indicating the existence of CsA and FK506’s targets other than calcineurin/NFAT pathway. Research has done to prove that CsA and FK506 can also inhibit an antigen specific Ca2+ independent pathway. Specifically, studies provided evidence that CNIs, in additional to Calcineurin inhibition, also block JNK and p38 pathways[6].

JNK and p38 falls into a superfamily called MAPK, an eukaryotic cascade participating in diverse biological processes, consists of MAPK, MAPK-K, MAPKK-K(MAPKK-K phosphorylates to activate MAPK-K, then MAPK-K goes through the same process to activate MAPK)[12]. Three distinct subgroups are categorised under the superfamily of MAPK: ERK, JNK, and p38. Although JNK and p38 primarily serve in stress responses[13], research has shown that both are activated when T-cell responses are triggered. The cooperative activation of JNK and p38, with ERK, leads to the activation of AP-1 and other transcription factors. Additionally, experiments have justified that by blocking JNK and p38 pathway, transcriptional activation of NF-AT cis-element was blocked. Therefore, since it was found that JNK and p38 are sensitive to CsA and FK506 activities, the drugs are predicted to prevent transcription of NF-AT cis-element[14].

Figure 2. Transcription of NF-AT cis-element.
3.4 FK506 has additional effects on IL-5 production and IL-7-induced T-cell proliferation. Despite the fact that CsA and FK506 has similar calcineurin/NFAT and MAPK pathways, significantly, FK506 has two other pathways. According to an unpublished finding found by Almawi et al., FK506 inhibits the activities of “i. IL-2 induced IL-5 production by human CD4+ T cells, and ii. T-cell proliferation stimulated by IL-2 and IL-7”. In contrast, CsA does not have the same ability[4].

Non-T-cell activities in immune system are affected by CsA and FK506 to different extents.

3.5 While both CsA and FK506 inhibit the release of histamine from basophils and the de novo synthesis of prostaglandin D$_2$ from mast cells, FK506 has a stronger impact on B-cell apoptosis[15][16]. FK506 and CsA both exhibit the ability to completely suppress the A23187-induced histamine release from basophils and the de novo synthesis of prostaglandin D$_2$ from skin mast cells. Possessing high affinity receptor for IgE, Basophils and mast cells are able to synthesise numbers of pro-inflammatory mediators and cytokines. Noticeably, two cells play essential roles in different phases and actions of cutaneous inflammatory reactions.

To prove that FK506 is able to inhibit histamine and prostaglandin synthesis, experiments were done by Amato de Paulis and his group. Data was collected and the hypothesis was confirmed.(See fig. 3) Similar experiments also show CsA has the same capability[17].

![Figure 3. FK-506 inhibit histamine and prostaglandin synthesis](image)

Nevertheless, although the mechanisms of FK506 and CsA as immunosuppressants share a great many of similarities, the drug effects vary in the aspect of B-cell apoptosis. Experiment was carried and the result shows a larger number of B-cell was apoptotic when treated with FK506. It shows that FK506 is more effective as a B-cell killer than CsA is.(See fig. 4)

![Figure 4. Drug effects in the aspect of B-cell apoptosis](image)

4. Applicable comparison between Cyclosporin A(CsA) and Tacrolimus (FK506)

4.1 Cyclosporin-Dosage
In case of adult organ transplantation, Cyclosporin is taken orally, and the drug dose varies depending on the transplant. For newly transplanted recipients, the following doses should be considered taken:
- Renal: 9 mg/kg/day (plus or minus 3 mg/kg/day) orally in 2 divided doses
- Liver: 8 mg/kg/day (plus or minus 4 mg/kg/day) orally in 2 divided doses
- Heart: 7 mg/kg/day (plus or minus 3 mg/kg/day) orally in 2 divided doses$^5$

4.2 Tacrolimus-Dosage
For adults who use Tacrolimus as immunosuppressant after the transplantation, the drug is orally taken and the recipients should take following as dosage guidance: “0.0375 mg per kg and 0.1 mg per kg
(0.017 mg per lb to 0.045 mg per lb) twice a day, with 12 hours between doses.” Notice that if the recipient’s cannot take FK506 orally, the drug may be given via intravenous injection.

4.3 Cyclosporin A and Tacrolimus (FK506) are neuroprotective, but Cyclosporin A itself also leads to skin cancer.

Despite the fact that FK506 and CsA are potent immunosuppressants, they are also discovered to function as neuroprotective drugs with different mechanisms. The mechanism of FK506 was not fully understood by scientists, but it was hypothesised that calcineurin activation can promote neural cell death because of the reason Calcium level. Therefore, by inhibiting calcineurin activity, FK506 is able to lower the death rate of neural cell, thus preventing neuro-diseases[18].

On the contrary, the pathway for CsA is much more complicated. MPTP (Mitochondrial Permeability Transition Pore) is a major factor associated with stroke-induced cell death and Parkinsonism. Noticeably, immunophilins (e.g. cyclophilin D) that bind to CsA also known as “neuroimmunophilin(within MPTP)”, are neuroprotective and can inhibit the function of MPTP. Since CsA interferes with immunophilin activity and the risk of getting neuro-diseases is observed to be lowered, it is then confirmed that CsA has the ability to inhibit the activity of MPTP[19].

Interestingly, through the same pathway, topical uses of CsA can induce skin cancer. MPTP, to explain in detail, is a “complex, multi-protein conductance channel” that can open in response to oxidative stresses. However, the introduction of CsA is proved to inhibit MPTP’s response. Experiment was done by using “UVA-irradiated HaCaT keratinocytes incubated with doses of CsA at therapeutic levels similar to those used in organ transplantation of 125 and 250 nM”. It was proved that CsA suppressed the apoptosis and necrosis of damaged cells, which, supposedly, will later spread and develop as cancerous cells, inducing skin cancer[20].

4.4 Cyclosporin A (CsA) has a greater impact on NO production, the leading factor of hypotension and nephrotoxicity.

As immunosuppressants, both CsA and FK506 tend to block the cytokine-induced NO production. However, because the two drugs carry out different mechanisms to block NO production, FK506 acts as a weaker suppressor than CsA for NO production. Since CsA is better at inhibiting NO production, it will also suppress the NOS2 gene expression. Considering the fact that NO production is “antihypotensive” and NOS2 helps the renal circulation of a recipient, CsA’s suppressive action upon NO production and NOS2 expression culminate in hypotension and nephrotoxicity[21].

5. Conclusion

CsA and FK506 are potent immunosuppressive drugs that are used to treat organ rejection. While both of them share cyclic structures, CsA and FK506 are synthesised using different synthetical strategies. Although CsA and FK506’s pathways share a surprisingly huge percentage of similarities immunosuppressively, FK506 was observed to be a significant stronger effect as an immunosuppressant. Meanwhile, CsA exhibit a few side effects induced by its unique MPTP pathway.

To settle the nephrotoxicity and hypotension among other side effects of Cyclosporin, new immunosuppressant called Fingolimod, with entirely different mechanistic pathways, is purposed. Hopefully, the drug will be introduced to the market in 2019.

References
[1] Gill, P, and L. Lowes. "The kidney transplant failure experience: a longitudinal case study." Progress in Transplantation 19.2(2009):114.
[2] “Information about Organ, Eye, and Tissue Donation.” Organ Donation Statistics: Why Be an Organ Donor?, HRSA, www.organdonor.gov/index.html.
[3] “Transplant Immunology.” British Society of Immunology. Sept. 2017.
[4] Almawi, W. Y., and O. K. Melemedjian. "Clinical and mechanistic differences between FK506 (tacrolimus) and cyclosporin A." Nephrology, dialysis, transplantation: official publication of
the European Dialysis and Transplant Association - European Renal Association 15.12(2000):1916.

[5] Wood, K. J., and R. Goto. "Mechanisms of rejection: current perspectives. " Transplantation 93.1(2012):1-10.

[6] Matsuda, S, and S. Koyasu. "Mechanisms of action of cyclosporine. " Immunopharmacology 47.2–3(2000):119-125.

[7] “Pharmacokinetics and Pharmacogenomics of Tacrolimus: a Review.

[8] Krönke, M, et al. "Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. " Proceedings of the National Academy of Sciences of the United States of America 81.16(1984):5214.

[9] Lawen, Alfons. “Biosynthesis of Cyclosporins and Other Natural Peptidyl Prolyl Cis/Trans Isomerase Inhibitors.” Biochimica Et Biophysica Acta (BBA) - General Subjects, vol. 1850, no. 10, 2015, pp. 2111–2120., doi:10.1016/j.bbagen.2014.12.009.

[10] Barreiro, C, and M. Martinezcastro. "Trends in the biosynthesis and production of the immunosuppressant tacrolimus (FK506). " Applied Microbiology & Biotechnology 98.2(2014):497-507.

[11] Harikishore, A, and H. S. Yoon. "Immunophilins: Structures, Mechanisms and Ligands. " Current Molecular Pharmacology 9.1(2015):-.

[12] Nishida, et al. "The MAP kinase cascade is essential for diverse signal transduction pathways." Trends in Biochemical Sciences 18.4(1993):128-131.

[13] Davis, R. J. "MAPKs: new JNK expands the group. " Trends in Biochemical Sciences 19.11(1994):470-473.

[14] Asami, T., et al. "Tacrolimus (FK506) inhibits interleukin-1β-induced angiopoietin-1, Tie-2 receptor, and vascular endothelial growth factor through down-regulation of JNK and p38 pathway in human rheumatoid fibroblast-like synoviocytes." Joint Bone Spine 79.2(2012):137-143.

[15] Bugliani, M, et al. "The direct effects of tacrolimus and cyclosporin A on isolated human islets: A functional, survival and gene expression study. " Islets 1.2(2009):106-110.

[16] Sehgal, V. N., G. Srivastava, and S. Dogra. "Tacrolimus in dermatology-pharmacokinetics, mechanism of action, drug interactions, dosages, and side effects: part I." Skinmed Dermatology for the Clinician 7.1(2008):27–30.

[17] De, Paulis A, et al. "Anti-inflammatory effect of FK-506 on human skin mast cells." Journal of Investigative Dermatology 99.6(1992):723-728.

[18] Muramoto, M, et al. "Detailed in vitro pharmacological analysis of FK506-induced neuroprotection." Neuropharmacology 45.3(2003):394-403.

[19] Osman, M. M., et al. "Cyclosporine-A as a neuroprotective agent against stroke: its translation from laboratory research to clinical application. " Neuropeptides 45.6(2011):359-368.

[20] Norman, K. G., et al. "Cyclosporine A suppresses keratinocyte cell death through MPTP inhibition in a model for skin cancer in organ transplant recipients." Mitochondrion 10.2(2010):94-101.

[21] Dusting, G. J., et al. "Cyclosporin A and tacrolimus (FK506) suppress expression of inducible nitric oxide synthase in vitro by different mechanisms. " British Journal of Pharmacology 128.2(1999):337-344.