CHAPTER II

REVIEW OF LITERATURE

I. Overview renal transplantation

Renal transplantation (RT) is the superior form of renal replacement therapy such as hemodialysis (HD) or continuous ambulatory peritoneal dialysis (CAPD), for end-stage renal disease (ESRD) patients[25]. The first successful human renal allograft transplant was performed between identical twins in 1954 at the Brigham and Women's hospital in Boston. In Thailand, renal transplantation have been started in 1972 at King Chulalongkorn Memorial Hospital.[38]

Graft survival depends on several factors including type and condition of the donor organ, type of surgical procedures involved, donor: recipient matches for human leukocyte antigen (HLA) alleles, and adequate immunosuppression after transplantation. Early graft function and freedom from rejection 6 months after transplantation are the most important independent prognostic factors for 5-year graft survival in recipients of primary cadaveric renal transplants.

Acute rejection episodes are associated with a significant increase in the risk of chronic graft rejection and graft loss. Currently, there is no effective therapy for chronic graft rejection; both immunological and nonimmunological factors appear to be involved in the ultimate impairment of organ function. Clearly, preventing or reducing the rate of acute rejection episodes will have a substantial impact on the rate of chronic rejection.

A. Immunological basis for acute graft rejection [26, 27]

1. Transplantation nomenclature

Graft	Any tissues, organ or object used for transplantation
Donor	The person who gave graft to the recipient

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Recipient	The person whom receive graft from the donor
Autograft	A transplant of tissue taken from the recipient such as skin's graft, hair's graft; not occur immune response
Syngraft (Isograft)	A transplant of tissue taken from identical gene donor; identical' s twins ;not occur immune response
Allograft	A transplant of tissue taken from the same species donor; the most common
Xenograft	A transplant of tissue taken from the different species donor such as porcine heart

2. Major histocompatibility complex (MHC) [26,27]

The major histocompatibility complex (MHC) is a glycoprotein, which address at the surface cells. Recognition between self versus nonself antigents.is the responsibility of MHC. In human, MHC is called the Human leukocyte antigent (HLA) and is located on the short arm of chromosome 6.

1. **MHC class I** consist of HLA-A, B, and C, which are expressed on the surface of all cells that have nucleus such as parenchymal cells within the allograft. These antigens promote the formation of antibody-producing B cells and are the primary target for cytotoxic T lymphocyte ($CD8^+$) reaction against allograft. Cytotoxic T cell recognize and respond to these antigents.

2. **MHC class II** consist of HLA –DP, DQ, and DR, which are only expressed on the surface of donor passenger antigent presenting cells (APCs), such as macrophage, dendritic cell, renal mesangial cell, kuffer's cell, alveolar type 2 lining cell, and B lymphocyte .MHC class II antgents trigger B cell generation and signal proliferation of T cells.

3. **MHC class III** do not play a specific role in the graft rejection process.

Before organ transplantation, histocompatibility testing and ABO blood group compatibility of the donor and recipient are mandated to minimize donor-specific immune responses to a transplanted organ.

3. Graft rejections

Three types of rejections are differentiated primary by histopathological features, but also by type of immune effector mechanisms that mediate the rejection process and by timing of occurrence.

1. Hyperacute graft rejection

Hyperacute graft rejection occurs immediately after transplantation, which antibodies respond to allogeneic MHC antigens and other antigents on the vascular endothelial cells within the donor organ. These antibodies fix complement, thereby promoting intravascular thrombosis. This causes rapid occlusion of the graft vasculature and leads to rapid rejection of the graft. Hyperacute rejection can be prevented by screening potential graft recipients for the presence of reactive antibodies.

2. Acute graft rejection

Acute rejection may occur weeks or months after transplantation. Two types of acute graft rejection division by tissue distribution. This one is acute vascular rejection, which is primarily mediated by pre-existing antibodies to donor endothelial antigents . Acute vascular rejection is characterized by necrosis of cells in the graft vasculature than thrombotic occlusion. Specific cytotoxic T-cells recognise and respond to alloantigents on the graft vasculature and include lysis of these cells. The second type is the acute cellular rejection, which is the most common form of graft rejection and is characterised by necrosis of parenchymal cells within the donor organ. Both T-cell ($CD4^+$ and $CD8^+$) and macrophages infiltrate the allograft. $CD8^+$ cytotoxic cells recognize and lyse allograft parenchymal cell, whereas

macrophages mediate delayed type hypersensitivity reactions. Cytokines produced by activated $CD4^+$ T-cells are responsible for sustaining the immune response by stimulating clonal proliferation of T-cells are recruiting inflammatory cells that cause endothelial necrosis. Natural killer (NK) cells also infiltrate the graft during acute cellular rejection and may kill allograft cells after recognizing foreign class I MHC antigens.

3. Chronic graft rejection

Chronic graft rejection occurs months or years after transplantation, which is characterized by intimal thickening and fibrosis, leading to luminal occlusion of the graft vasculature. Although the pathophysiology of chronic rejection is not completely understood, repetitive injury of the graft vacular endothelium results in increased expression of adhesion molecules, which in turn activates leukocytes. Subsequent release of cytokine such as IL-2, IL-6, transforming growth factor-beta (TGF-β) lead to obliteration of the blood vessels as well as to glomerulosclerosis.

4. Immunosppressive agents[27]

The development of immunosuppressive agents paralleled the progress in the understanding of cellular and molecular mechanisms of allograft rejection. Thus, initial experiments early in the century, using the cytotoxic agents (benzene, toluene and irradiation), paved the way for the use of relatively nonselective antiproliferative agents (azathioprine [AZA], cyclophosphamide)

In 1959, by using the anticancer drug 6- mercaptopurine (6-MP), Schwartz and Demeshek showed that pharmacologic immunosppression was possible after transplantation, but clinically limited by the requirement for parenteral administration. Two years later, AZA, an orally absorbed analog of 6-MP, was successfully used for human allograft transplantation by Calne initiating the modern era of transplantation. In the early 1960s, AZA and corticosteroids provided the basis of standard immunosuppressive therapy in transplantation. In 1983, cyclosporin (CSA) has been approved by the US Food and Drug administration (USFDA), which importancely

advanced short-term RT outcomes and permitted successful transplantation of other organs, such as the heart, lung, liver, and pancreas. In 1975, using the somatic cell hybridization technique developed by Kohler and Milstein, monoclonal antibodies directed to the antigens expressed on the surface of the T-cells. A full decade later, an explosion of new agents including mycophenolate mofetil (MMF), which approved by USFDA in 1995, tacrolimus (FK 506), sirolimus, which acted on postreceptor signal transduction pathways, and genetically engineered humanized interleukin-2 (IL-2) antibodies.

In general, immunosuppressive therapy in organ transplantation has 2 periods are called the induction therapy, which is the aggressive immunosuppresion period and the maintenance therapy, which is sustain immunosuppression for prevention of graft rejection, but also the rescue therapy for the reversal or treatment of graft rejection.

1. Azathioprine (AZA)

Mechanism of action

AZA is a competitive inhibitor of both de novo and salvage enzyme pathways of nucleotide synthesis. AZA is converted intracellularly to thioinosinic acid monophosphate (TIMP), which manifests four pharmacological effects: (1) it inhibits the activity of inosine monophosphate dehydrogenase (IMPDH), a critical enzyme in the de novo pathway to generate purine nucleosides adenylic or guanidylic acid; (2) TIMP also blocks phosphoribosyl-pyrophosphate aminotransferase, the first step in the de novo synthesis of purines; (3) as a structural analog of IMP , it activates a negative feedback circuit which limits production of IMP from its precursors ; and (4) 6-thioguanine (TGN), the primary active metabolite of AZA, are directly incorporated nucleic acids as fraudulent bases. The consequent chromosomal breakage, nucleic acid distortion, and interference with DNA repair produce and increased proclivity of drug-treated cells toward malignant transformation as well as benign papillomatosis.

Pharmacokinetic properties

Approximately 50 % of AZA is absorbed after oral administration. A daily oral dose of 2-3 mg/kg is generally administered when the agent serves as the primary immunosuppressant, and a dose of 1-2 mg/kg when it is used in adjunctive therapy with CSA. Dose adjustment is often not necessary in cases of renal dysfunction because the drug is neither significantly dialyzed nor excreted by the kidney; however, the AZA dose should be reduced by 25 % to 50 % when it is coadministered with allopurinol which also inhibits xanthine oxidase activity.

Clinical efficacy and toxicity

A number of investigates documented beneficial effects of an AZA/Pred combination therapy, which became the " conventional" immunosuppressive protocol between 1966 and 1978. The AZA and steroid combination was clouded, however, by two major dose-related side effects: bone marrow suppression and defects in metabolism and wound healing, respectively. This regimen displayed a therapeutic index; about 85 % of renal allograft recipients experienced acute rejection episodes and only 50 % of grafts survived a full year. The limitations of AZA led to an interest in the development of nucleoside synthesis inhibitors which could act noncompetitively and more selectively to inhibit purine synthesis.

3. Mycophenolate mofetil (MMF)

MMF, a synthetic derivative of MPA produced by the mold *Penicillium glaucum*, was approved and deemed superior to AZA, which will refer to II. MMF.

4.Cyclosporin (CSA)

Mechanism of action

CSA inhibits transcription of the IL-2 gene by inhibiting calcineulin phosphatase activity. CSA is converted to active formulations by binding to, and undergoing structural conversion by, cytoplasmic propyl peptidyl isomerases namely cyclophillin

(Cyp). This complex bind to the phosphatase CaN to form a pentameric unit, which leads to inhibition of calcium-calmodulin-dependent calcineulin activity , thus preventing the dephosphoration of nuclear factors of activated T-cells pre-exiting (NF-ATp) by calcineulin and the subsequent translocation of NF-ATp to the nucleus. Because all the factors required for transcription of the IL-2 gene must be present before any of them can bind to the promotor region of the IL-2 gene, CSA effectively shuts down expression of the IL-2 gene by preventing the translocation of NF-ATp.

Pharmacokinetic properties

Adminstered orally, the bioavailability of CSA ranges from 50 % to 60 %. CSA is extensively distributed throughout the body. Two-thirds of the CSA in blood is bound to erythrocytes, and the other third to plasma lipoproteins; thus, the drug is not significantly dialyzable. The parent compound has a half-life of about 8 hours. CSA is primarily metabolized by cytochrome P-450 3A4 isoenzymes, with the majority of metabolites excreted in the bile and only a small amount via the renal route. Thus, in renal dysfunction, dose modification is usually not necessary during dialysis therapy. However, hepatic dysfunction reduces drug clearance. The combination of CSA and TCL also potentiates the toxic side effects of the individual drugs, particularly nephrotoxicity. Monitoring CSA exposure is best achieved by measuring the average concentration of the drug (Cav), the quotient of the area under the plasma concentration-time curve (AUC), and the dosing interval in hours. Low levels of exposure are associated with the occurrence of acute rejection episodes, and high intraindividual variability in drug exposure with an increased risk of chronic rejection. The most popular method for estimating CSA is the automated TDx assay.

Clinical efficacy and toxicities refers to TCL.

5.Tacrolimus (TCL, FK506)

Mechanism of action

Mechanism of action of TCL like a CSA. TCL binds to FK binding proteins (FKBP) while CSA binds to cyclophillin.

Pharmacokinetic properties

Administered orally, the bioavailability of TCL ranges from 4% to 93 % (mean of 25%). TCL concentrations are 11 to 114 times higher in whole blood than in plasma. TCL is metabolized by cytochrome P-450 3A4 isoenzymes, like CSA. Optimal efficacy was observed among patients who displayed TCL trough concentrations of 5 to 15 ng/mL Trough concentrations below this range were associated with a 30 % incidence of acute rejection episodes, and those above, with a 45 % incidence of adverse events and a 3 % incidence of rejection episodes. CSA displays narrower pharmacokinetic variability than TCL.

Clinical efficacy and toxicities

The TCL treated group displayed a significantly reduced incidence of acute rejection episodes compared with the group receiving CSA, but the overall patient and graft survival rates were similar. CSA and TCL appear to have no differences in their short-or long-term therapeutic results. Furthermore, TCL/MMF- and CSA/MMF-based immunosuppressive regimens yield comparable patient and graft survival among renal transplant recipients. Thus, the selection of a calcineulin inhibitor is generally based on physician preference, on patient intolerance to one of the alternated drugs, for example, because of cosmetic side effects of CSA or neurotoxicity of TCL, on ill-defined pharmacodynamic resistance, and/or on idiopathic nephrotoxic sensitivity. The most common adverse events associated with TCL therapy are neurotxicity (tremor, seizures, coma, and headache), nausea and diarrhea, alopecia, as well as new onset posttransplant diabetes mellitus (PTDM). The incidence of PTDM is significantly higher among TCL than CSA –treated patients (19% versus 4 % respectively; $p< 0.001$). Reports on the incidence of hypertension and lipid disorders under TCL therapy are inconsistent.

6. Sirolimus (SLM)

SLM is a macrocyclic lactone product of *Streptomyces hygroscopicus,* discovered in the soil of the Vai Atari region of RapaNui (Easter Island). The drug not only prolongs allograft survival, but also interacts synergistically with CSA.

Mechanism of action

SLM blocks cytokine-driven cell proliferation and maturation by inhibiting a multifunctional phosphatidyl-inositol kinase-mammlian target of rapamycin (mTOR) regulates the phosphorylation status of several sarcomar-like, receptor-type, and cell-cycle-dependent kinases, as well as intracellular phosphatases. SRL blocks Ca^{2+} - dependent and Ca^{2+} - independent events, including transduction of the signals delivered by IL-2, IL-4, and IL-15. To a lesser degree, SRL blocks signals delivered by nonlymphoid cytokines: fibroblast growth factor, stem cell factor, platelet-derived growth factor (PDGF), colony stimulating factor (CSF), and insulin growth factor (IGF). Finally, SLM inhibition prevents hyperphosphorylation of retinoblastoma protein, thereby reducing E2F-dependent DNA transcription and decreasing Bcl-2 activity so as to promote apoptosis.

Pharmacokinetic properties

SLM exhibits an oral bioavailability of about 15 % and an average terminal half-life between 57 to 62 hours in stable renal transplant recipients receiving CSA. An excellent correlation (r=0.94) exits between the trough whole-blood levels and the AUC , after multiple drug doses, suggests that the trough level is a good indicator of total drug exposure. SLM is extensively distributed in tissues, with a volume of distribution of 5.6 to 16.7 L/kg in stable renal transplant recipients, which permits once daily dosing [39] Similar to TCL and CSA , SLM is metabolized to some extent in the small intestine and extensively in the liver via by cytochrome P-450 3A4 isoenzyme, and is countertransported in the gut lumen by the multidrug efflux pump, P-glycoprotein ; these processes account for low bioavailability and high pharmacokinetic variability. The rate and extent of oral absorption of SLM is reduced in black patients. Total body clearance 127 to 240 mL/hr/kg, which is not related to dose. The major of excretion is feces, 91 %.

Clinical efficacy and toxicity

When used as the foundation of immunosuppression, a regimen of SLM/AZA/Pred showed a comparable incidence of acute rejection rates at 6 months as a CSA/AZA/Pred regimen; namely, 41% and 38 %, respectively in a phase II open-label trial. In contrast, the addition of SLM to a CSA/Pred regimen reduced the incidence of

acute rejection episodes (from 36 % to 7 %) and permitted early withdrawal (within 1 month) of steroid therapy in 78 % of patients. The most common side effects associated with SLM therapy are thrombocytopenia, leukopenia, and hyperlipidemia (40 %).

7.Polyclonal antibodies

Polyclonal antibodies preparations directed against immunocomponent cells have been used successfully to prevent or reverse acute rejection episodes. Polyclonal antibodies preparations are used to deplete or modulate the activities of T and/or B cells. Two polyclonal reagents: equine ATGAM, which is horse antibodies to human thymocytes and rabbit thymoglobulin (ALG), which is rabbit antibodies to human lymphocytes, became available.

Mechanism of action

These antibodies bind to cell surface receptors, thereby opsonizing lymphocytes for complement-mediated lysis or reticuloendothelial cell-dependent phagocytosis.

Dose and administration

ATGAM :

A daily dose of 10 to 20 mg/kg/day of ATGAM is usually infused over 4 hours through an in-line filter into a central venous line catheter or arteriovenous fistula because the use of a peripheral vein is often followed by vein thrombosis or thrombophlebitis. The course of polyclonal antibodies needed to reverse moderate or severe acute rejection episodes is 10 to 21 days.

ALG:

Thymoglobulin is infused over 6 hours peripherally at a dose of 1.5 mg/kg daily for 10 to 21 days.

Clinical efficacy and toxicity

The incidence of adverse events and the 1-year patient and graft survival rates were similar in both groups. Many of the side effects of these treatments (chills, fever, and arthralgia) were related to the large quantities of administered foreign protein. Thrombocytopenia and leukopenia represented not uncommon side effects. The other side effects of these reagents related to the intense T-cell inhibition which increased the incidence of lymphomas and opportunistic infections, particularly with CMV, so as to necessitate prophylactic antiviral antibiotic treatment.

8. Monoclonal antibodies

In contrast to polyclonal antibodies, a monoclonal antibodies preparation is highly specific, recognizing a single epitope on its target antigent. Monoclonal antibodies are produced by hybridomas generated by the fusion of nutrient-dependent plasmacytoma cells which contribute immortality with spleen cells from mice, which have been immunized to human lymphocytes and are producing a single antibody of designed specificity.

- **Muromonab CD3 (Orthoclone OKT3)**

Mechanism of action

OKT3 is a murine monoclonal antibody of the IgG_{2a} class, binds to the epsilon chain of the CD3 receptor on T lymphocytes, causing modulation and immunological inactivation of CD3+cells. It is believed that OKT3 produces a cell surface modulation of shedding, endocytosis, or blindfolding of the CD3 marker. One obstacle to therapy is the production, by 50% of treated patients, of neutralizing antiidiotype human anti-murine antibody (HAMA), as a result, most mouse monoclonal antibodies have a half-life of only 1 to 2 days, leading to a patient resistant to repeated courses of therapy.

Clinical efficacy and toxicity

Currently, OKT3 is administered for induction therapy, as well as for reversal of severe acute or steroid-resistant rejection episodes. Even though pan T-cell-specific

monoclonal antibodies have been used effectively to treat graft rejection, there are some risks associated with their use. Cytokine release syndrome is frequently a serious adverse event associated with the use of OKT3 and is characterized by a massive, albeit transient, release of T-cell cytokines, including tumor necrosis factor-alpha (TNF-α), interferon-gamma, IL-2, IL-3, and IL-6. Symptoms of cytokine release syndrome include fever (73%) , chill (57%), tremors(10%), dyspnea (21%), chest pain/tightness (14%), wheezing (11%), nausea (11%), and/or vomiting (13%). Strategies to minimize the occurrence of these adverse effects include premedicating a patient with a cocktail of methylprednisolone (5 to 8 mg/kg), diphenhydramine HCl (25 to 50 mg IV), and acetaminophen (500 mg orally), or using a low initial dose of antibody (1 mg) followed by gradual escalation of the amount to the usual 5 mg dose.

- **Interleukin-2 receptor monoclonal antibody (IL-2R mAb) reagents**

Basiliximab and daclizumab are two anti-IL-2R mAb reagents recently approved for prophylaxis of acute rejection episodes in renal transplantation. They still represent important additions to the induction regimen. Therefore, physicians may use the anti-IL-2R mAbs for induction therapy, reserving the use of OKT3 or ATGAM/thymoglobulin for potential subsequent rejection episodes.

Mechanism of action

anti-IL-2 receptor mAbs bind to the alpha chain (CD25) of the IL-2R, which cannot trigger cellular activation; these antibodies do not elicit the cytokine release syndrome. Furthermore, both of the anti-IL-2R mAbs display a prolonged serum half-life and rarely evoke the production of neutralizing antibodies. Blockade of IL-2R inhibits T lymphocye clonal proliferation and differentiation.

Dose and administration

Basiliximab

The chimeric form, basiliximab mAb, displays a 10-fold greater avidity for IL-2R than daclizumab, ahumanized version of the murine anti-Tac mAb. Basiliximab is administered intravenously on the day of the surgery and on the fourth day

posttransplant. Anti-IL-2R mAbs produce a blockade of IL-2R, beginning at 10 hours after the initial dose and lasting as long as 4 months posttransplant.

Daclizumab

Daclizumab is administered intravenously at 1 mg/kg/day beginning within 24 hours after transplantation, followed by equal doses very 14 days for a total of 5 doses.

Clinical efficacy and toxicity

Two multicenter trials have documented that administration of two 20 mg doses of basiliximab with concomitant CSA and steroid therapy produced results; a decreased incidence (29.8 % for basiliximab treatment versus 44 % for placebo ;P=0.012) as well as severity of acute rejection episodes. The addition of daclizumab to a triple-drug regimen of CSA, AZA, and steroids significantly reduced the incidence of biopsy-proven acute rejection within the first 6 months; namely, 22% for daclizumab treatment versus 35% for the placebo (p=0.03).

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Table 1 Immunosuppressive agents utilized most frequently in transplantation [26,27]

Category ; Agents	Mechanism of action	Usual Dose	Adverse Effect	comments
Corticosteroids				
Methylprednisolone Prednisolone Hydrocortisone	Inhibit T-cell proliferation and cytokine gene transcription, anti-inflammatory properties	Initial prednisolone; 0.5-2 mg/kg/day po Maintenance prednisolone; 0.1-0.2 mg/kg/day po	osteoporosis, suppression of adrenal function cushing noid facies, , acne, DM, hyperlipidemia, growth retardation, hypertension, cataracts, fluid retention, delayed wound healing	Take immediately after meals to minimize GI side effects
Interleukin-2 Inhibitors				
Cyclosporin (Sandimmune-Neoral; Novartis)	bind to cyclophillin; inhibit T-cell proliferation	Initial;4-12 hr before surgery; 5-6 mg/kg/day Ivor 14-18 mg/kg/day po Maintenance: 5-15 mg/kg/day po divided every 12-24 hr adjusted dose by whole blood trough levels and adverse effects	Hyperclipidemia, hirsutism, gingival hyperplasia, nephrotoxicity,hypertension, neurotoxicity (tremor), hyperglycemia, hyperkalemia, hyperuricemia, hypomagnesemia	IV slow infusion 2-24 hr ;Glass bottles only administration of IV or oral solution
Tacrolimus (FK506; Prograft; Jansen-Cilag)	bind to FKBP; inhibit T-cell proliferation	InitiaL; 0.05-0.1 mg/kg/day IV is given at least 6 hr after surgery Maintenance; 0.15-0.3 mg/kg/day po divided every 12 hr ,adjusted dose by trough and adverse reactions	PTDM, alopecia, neurotoxicity (tremor),hypertension, nephrotoxicity, hyperlipidemia	Trough levels 5-15 ng/mL

Table 1 Immunosuppressive agents utilized most frequently in transplantation (continue)

Category ; Agents	Mechanism of action	Usual Dose	Adverse Effect	comments
Interleukin-2 Inhibitors				
Sirolimus (apamycin, rapamune)	inhibit signal transduction; inhibit T-cell proliferation	Loading dose; 6 mg po once daily Maintenance dose; 2 mg po once daily	hyperlipidemia (40%), leukopenia, thrombocytopenia impair erythroid recovery, anemia	Before taking the dose, it should be diluted with water or orange juice; but not be diluted with grapefruit juice
Antimetabolites	inhibit T and B cell proliferations			
Azathioprine (Imuran; Glaxo-Wellcome)	inhibit both a *de novo* and *salvage* pathways; inhibit B and T-cell proliferation	Initial; 2-5 mg/kg/day po Maintenance; 1-2 mg/kg/day po	bone marrow suppression; hepatotoxicity, fever,chill,N/V, anorexia, infection	Decreased 25-50 % of dose when administered with allopurinol
Mycophenolate mofetil (CellCept; Hoffman-La oche)	inhibit de novo pathway; inhibit B and T-cell proliferation	500-1500 mg po every 12 hr	gastrointestinal adverse events; diarrhea, abdominal pain, infection; CMV ,leukopenia	Avoid to administration with antacid, Fe preparation cholestyramine, ASA, probenecid
Polyclonal : Antibodies	bind to cell surface receptors, complement-mediated lysis or reticuloendothelial cell-dependent phagocytosis			
		escute therapy		
Antithymocyte globulin (equineATGAM, Pharmacia-Upjohn)		10- 20 mg/kg/day IV infusion over 4 hr for 10-21 day	chills,fever,arthralgia, lymphomas, opportunistic infections	Infusion through an in-line filter into a central venous line catheter or arteriovenous fistula

Table 1 Immunosuppressive agents utilized most frequently in transplantation (continue)

Category ; Agents	Mechanism of action	Usual Dose	Adverse Effect	comments
		escute therapy		
Thymoglobulin (rabbit; Sangstat)		1.5 mg/kg daily IV infusion over 6 hr through peripheral for 10-21 day	chills,fever,arthralgia, oppoistic i lymphomas,	
ections				
Monoclonal:Antibodies				
Muromonab-CD3 (KT3; rthoclone; rtho Pharmaceutical)	bind to CD3-receptor on T lymphocytes; inhibit thymus-derived lymphocytes	escute therapy or Induction therapy; 5 mg/day IV for 5-14 day	"cytokine release" syndrome;fever,chills; tremor, dyspnea,wheezing, chest pain,nausea or vomiting	Administered undiluted over 1 min; filter through a 0.22 micron filter
Interleukin-2 eceptor Monoclonal:Antibody (IL-2 Mab) eagents Bind to the alpha chain (CD25) of the IL-2 ; inhibits T lymphocyte clonal proliferation and differentiation				
Basiliximab (CHI 621 Simulect; Norvatis)		Induction therapy 20 mg IV on the day of the surgery and on the fourth day		
Daclizumab (Zenapax; Hoffman-La oche)		1 mg/kg/day IV within 24 hr of transplant surgery then equal doses every 14 days for a total of 5 doses		

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II. Mycophenolate Mofetil (MMF)

A. Pharmacodynamic Properties

Mycophenolate Mofetil (MMF) is an immunosuppressive agent introduced into clinical practice in 1995 for the prevention and treatment of allograft rejection. More recently, it has also been used for the treatment of autoimmune disease example systemic lupus erythematosus (SLE). MMF is a prodrug that is rapidly hydrolysed by esterases to the active compound mycophenolic acid (MPA). In turn, MPA is metabolized to the beta-glucuronide. MPA and the beta-glucuronide are interconverted during enterohepatic circulation resulting in only 10-20 % of the drug being in the active form, but the half-life (T ½) of the active compound is extended.

MPA was initially isolated from the mould *Penicillium brevicompactum* in the 1940s[1,2], and studied at the beginning of the 1970s for the treatment of refractory psoriasis and cancer[3].

The main mechanism of action of MPA is the inhibition of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* pathway of purine synthesis[4], which is required for the proliferation and function of T and B lymphocytes[5,6]. Since T and B lymphocytes rely soley on this pathway for the production of guanosine nucleotides, the proliferation of these cells is specially inhibited[7]. In addition, further modes of action exist[8] two isoforms of IMPDH have been identified with different expressions and sensitivity to MPA. MPA potently, selectively and reversibly inhibits IMPDH. Unlike most other cells, lymphocytes rely on the *de novo* pathway more than the *salvage* pathway (hypoxanthine- guanine phosphoribosyltransferase) for purine biosynthesis. Enterocytes rely approximately 50 % on the *de novo* pathway; however this may change during long term therapy with drugs such as MMF[9].

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B. Pharmacokinetic Properties [32-35]

Absorption

The pharmacokinetics of the immunosuppressant mycophenolate mofetil (MMF) have been investigated in healthy volunteers and mainly in recipients of renal allografts. Following oral administration, MMF was rapidly and completely absorbed, underwent extensive presystemic de-esterification. Bioavailability (F) of oral MMF was around 94.1 %[1]. In renal transplant recipients, very low serum level of MPA were achieved after oral MMF therapy in the early post transplantation period; serum levels increased significantly after 20 days of treatment, suggesting potentially impaired absorption or altered metabolism of the ester in uremic patients. A pharmacokinetic study showed that the rate but not the extend of absorption of MMF is affected by food, indicating that MMF may be administered with food. However, Prod. Info. CellCept ®(1998) suggested that the dose of MMF should be administered on an empty stomach.

Distribution

Total protein binding of MMF was around 97 %. The magnitude of the MPAG renal clearance indicates that active tubular secretion of MPAG must occur. At clinically relevant concentrations, MPA and MPAG are about 97 % and 82 % bound to albumin, respectively. Thereby, MPAG at high (but clinically realizable) concentrations reduced the plasma binding of MPA. In patients with renal impairment or delayed graft function (DGF), protein binding may be decreased . In addition, volume of distribution (Vd) was around 3.6 to 4 L/kg.

Metabolism

In healthy volunteers systemic plasma clearance (Cl) of intravenous MMF was around 10 L/min and the half-life (T1/2) was around 2 minutes, and plasma MMF concentrations fell below the quantitation limit (0.4 mg/L) within 10 minutes of the cessation of infusion. MMF is rapidly converted to its active form, mycophenolic acid (MPA), by inosine monophosphate dehydrogenase (IMPDH) in the liver. MPA is

subsequently metabolized to mycophenolic acid glucuronide (MPAG), which is inactive form. MPAG is excreted in the bile. Deconjugation of the glucuronide to MPA may occur in human via the action of intestinal or intestinal microflora beta – glucuronidase, which subsequent reabsorption of MPA. An oral cholestyramine interaction study showed the mean contribution of enterohepatic cycling to the AUC of MPA was around 40 % with a range of 10 to 60 %.

A secondary rise in the plasma concentration of MPA occurred with both intravenously and orally of administration 8 to 12 hours after the dose[1]. Thereby, after administration of MMF 1.5 g intravenously and orally, the apparent half-life was 16.6 hours and 17.9 hours, respectively.

Excretion

Renal excretion of MPAG was 93 %, for MPAG is recovered in the urine while the other excretion was around 6 % by feces. It is not known whether MPA is excreted in human milk. The apparent clearance (Cl) was approximately 180 mL/min.[1] Similar plasma MMF concentrations were seen after intravenous administration in patients with severe renal or hepatic impairment, implying that the de-esterification process had not been substantially affected.[11]By the way Johnson HJ,et al.[34] have been investigated in patients with varying degrees of renal function .They have been discovered that clearance of MPA after a single 1 gm oral MMF is unaffected by renal function. Changes in renal function after transplantation should not result in significant differences in elimination of MPA. In addition, because MPA minimally removed by hemodialysis, MMF may be administered without regard to hemodialysis after kidney transplant in patients who continue to require intermittent dialytic support. Supplemental doses of MMF are not required after hemodialysis. On the other hand, MPAG clearance as glomerular filtration rate (GFR) is affected by renal function. Compared to individuals with a normal GFR, patients with severe renal impairment (GFR 1.5 L/hr/1.73 m^2) showed 3- to 6- fold higher MPAG AUC values. It is unclear whether accumulation of MPAG is associated with side effects or an overall increase in exposure to active MPA

C. Therapeutic drug monitoring (TDM)

The use of TDM as a tool to assist the very important process of regulating the exposure of patients, thereby facilitating their safe and effective use. The mean maximum plasma MPA concentration (Cmax) after a MMF 1 gm dose in healthy individuals was around 25 mg/L, occurred at 0.8 hours post dose, decayed with a mean apparent T1/2 of around 16 hours, and generated a mean total area under the plasma concentration-time curve (AUC) of around 64 mcg.hr/mL. Pescovitz MD et al [35] have been investigated and suggested that the MPA AUC (0-12) was higher for intravenous MMF than oral MMF (40.8 $\pm$ 11.4 vs 32.9 $\pm$ 15 mcg.hr/mL, $p < 0.001$). But there were no other significant pharmacokinetic difference for plasma MPA or MPAG. Compared with MPA, MPAG showed a roughly similar Cmax about 1 hour after MPA Cmax, with a similar T1/2 and an AUC about 5-fold larger than that for MPA.

D. Drug interactions [14, 36]

1. Cholestyramine

Plasma concentrations of MPA may be significantly reduced when coadministered with cholestyramine. The consequent, this interaction may be reduced MMF efficacy. Twelve healthy volunteers pretreated with cholestyramine 4 g three times daily for four days had a decreased AUC of MPA following a single dose of MMF 1.5 g. This interaction is though to be due to an interruption of the enterohepatic recirculation due to binding of MPAG with cholestyramine in the intestine . So it is recommended that MMF and cholestyramine not be coadministered.

2. Antacids

MMF absorption may be decreased when administered concurrently with antacids containing magnesium hydroxide and dose of MMF 2 gm with Maalox ® TC 10 mL, the Cmax and AUC for MPA decreased by 33% and 17%, respectively. Doses of MMF and antacids containing magnesium and aluminum should be staggered. The consequent, this interaction may be reduced MMF efficacy. Should it be necessary to use this

combination, stagger the dosing in patients receiving MMF and antacids containing magnesium hydroxide and aluminum hydroxide.

3. Iron ion preparations

Megumi M et al (2000) [36] reported that when MMF and iron ion preparations were administered concomitantly, a remarkable decrease of MMF absorption (89.7 %) was observed. This study designed as a randomized crossover, in 7 healthy volunteers, they found the AUC from 0 to 12 hours and the Cmax of MPA in MMF combined with iron ion preparations were significantly less than in MMF alone (MPA AUC, 32.9 $\pm$ 14.7 mcg*hr/mL versus 2.92 $\pm$ 0.883 mcg*hr/mL , $p < 0.01$, Cmax, 20.1 $\pm$ 9.21 mcg/mL versus 1.30 $\pm$ 0.367 mcg/mL, $p< 0.01$). It is suggested that the interaction between MMF and iron ion is caused by impairment of drug-iron ion complexes. MMF contains 4-hydroxy radical and 3-oxo-benzofuran. The mechanism of the interaction between MMF and iron ion probably is the formulation of the chelation complexes between this radical and iron ion. The resulting complexes have poor gastrointestinal absorption. This interaction between MMF and iron ion is similar to the interaction of tetracycline and iron ion or cedinir and iron ion, and this impairment by iron ion on the absorption of MMF was more pronounced than that by metal cation on ciprofloxacin or ofloxacin. Therefore it seems to be clear that we must avoid the concomitant administration of MMF and iron ion preparations.

4. Cyclosporin (CSA)

In a study in heart transplant patients, Ensley et al (1993) report that coadministered cyclosporin and MMF appeared to be safe and well-tolerated without significant renal or hepatic toxicity, even when given long term (430 $\pm$ 30 days) in heart transplant patients. Serum CSA concentrations and doses showed no significant changes during combined therapy with oral MMF. In a preliminary study involving 48 renal transplant patients, Sollinger et al (1992) found a lack of significant toxicity when MMF, CSA and prednisone were coadministered. One patient developed hemorrhagic gastritis. However, Van Gelder T, et al, reported that the MPA concentrations in non-CSA-treated

patients were significantly higher, possibly due to increased glucuronidation in the CSA group as a result of CSA induced induction of the cytochrome P-450 complex.

5. Sulfinpyrazone

Catalano et al (1997) reported increased MMF toxicity in an anoroexic, 50 year-old female, renal transplant patient also treated with sulfinpyrazone. Even though the dose of MMF was adjusted for the level of renal function, the patient developed signs of toxicity, including nausea, diarrhea, and abdominal pain. Signs of toxicity resolved after both drugs were discontinued. Sulfinpyrazone may have interfered with the renal tubular secretion of MMF.

E. Adverse drug reactions [6, 16]

1. Gastrointestinal adverse effects

Diarrhea has been reported in 31 % to 36 % of renal transplant patients in clinical studies; constipation has occurred in approximately 18% to 23 % and nausea occurred in approximately 19% to 24 % of patients. Nausea and diarrhea, usually occur early in therapy and respond to dose reduction or switching from two to three divided daily doses. Abdominal pain, and dyspepsia (occurring in 13 % to 17 % of renal transplant patients. Hemorrhagic gastritis has been reported in one renal transplant patient, which resolved after withdrawal of treatment. Pancreatitis was described in 1 renal transplant recipient during rescue therapy with MMF. Resolution of symptoms occurred after discontinuation of treatment. The incidence of adverse gastrointestinal complications requiring dose reduction (or more rarely discontinuance of therapy) has ranged from 5% to 20% in renal and heart transplant patients. However, Epinette et al (1987) reported that all gastrointestinal adverse effects diminished significantly during subsequent years of therapy. The aetiology of the gastrointestinal adverse effects of MMF is still not completely clear. It is a combination of several effects.[6]

2. Hematologic effects

In controlled clinical studies, leukopenia has occurred in 11 % to 34 % of transplanted patients receiving MMF; anemia has occurred in 25 % to 43 %. The incidence of the above adverse effects varied with the MMF dose, study, and type of transplant. Thrombocytopenia has occurred in 8 % to 24 %. In addition, hypochromic anemia and leukocytosis occurred in approximately 7 % to 25 % and 7 % to 40 % of patients. Mild leukopenia and anemia (but not thrombocytopenia) have been reported rarely in rheumatoid arthritis patients treated with oral MMF. During long-term therapy of psoriasis with oral MMF, leukopenia has also occurred, and has occasionally been dose-limiting; mild anemia and thrombocytopenia have generally been less frequent. The myelosuppressive effects of MMF are less than those of azathioprine. Substitution of MMF for azathioprine as antirejection therapy in heart transplant patients has been associated with significant increases in hematocrit and absolute neutrophil counts.

3. Cardiovascular effects

Hypertension has occurred in 17 % to 32 % of renal transplant patients in clinical trials; peripheral edema has occurred in approximately 28 % to these patients. After cardiac transplantation, the incidence of hypertension, hypotension, and peripheral edema was 77.5 %, 32 % and 64 %, respectively.

4. Hepatotoxicity

Elevation of liver enzymes has been reported occasionally in some studies. However, no effect on liver function has been reported in most.

5. Central nervous system effects

Headache has occurred in 16 % to 21 % of renal transplant patients in clinical trials. In addition tremor was reported in 11 %, insomnia in 9 % to 11 %, and

dizziness in 5 % to 11 %. After cardiac transplantation, the following adverse effects were reported: headache (54.3 %), insomnia (40.8 %), dizziness (28.7 %), anxiety (28.4 %), tremor (24.2 %), and paresthesia (20.8 %). In patients with rheumatoid arthritis, headache, dizziness insomnia, and weakness have been described occasionally. With relatively high doses of oral MMF for the treatment of psoriasis, weakness, headache, and insomnia were observed in 20 %, 5 %, and 11 % of patients, respectively, during the first year of treatment. These effects diminished significantly during subsequent years of therapy.

6. Endocrine and metabolic effects

Hyperglycemia has been reported in 8 % to 12 % of renal transplant patients while hypercholesterolemia, hypophosphatemia, hypokalemia, and hyperkalemia have occurred in approximately 8 % to 15 %.

7. Genitourinary effects

Urinary tract infection occurred in 37 % of renal transplant patients in clinical trials. Hematuria was reported in 12 % to 14 % of patients and kidney tubular necrosis was reported in 6 % to 10 %. After cardiac transplantation, elevations of serum creatinine and blood urea nitrogen were reported in 39 % and 34 % of patients. Burning on urination and urinary frequency or urgency were reported in 16 % of psoriasis patients during the first year of MMF treatment in one study. Oral MMF has occasionally been associated with urinary frequency.

8. Ocular effects

Oral therapy with relatively high doses of MMF in psoriasis patients has been associated with ocular changes, including nuclear sclerotic cataracts, blepharitis, keratitis, glaucoma, and macular abnormalities. These complications have not been reported in heart or renal transplant recipients treated with MMF.

9. Respiratory effects

In clinical trials involving renal transplant patients, respiratory infection occurred in 22 % to 24 %, dyspnea occurred in 15 % to 17 % of patients, increased cough occurred in 13 % to 15 %, and pharyngitis occurred in 9 % to 11 % of patients. After cardiac transplantation, the following adverse effects were reported: infection (37 %), dyspnea (36.7 %), cough (31.1 %) and sinusitis (26 %).

10.Rash

Rash occurred in 6 % to 7 % of renal transplant patients treated with MMF; acne has occurred in approximately 10 %. After cardiac transplantation, rash was reported in 22 % of patients.

11.Musculoskeletal effects

Leg cramps or pain, bone pain, myalgias, and hand cramps have been reported occasionally during MMF administration.

12.Other

◊ Adverse effects-general

A variety of infections related to immunosuppression have been observed in renal and heart transplant recipients during MMF dosing, including cytomegalovirus (CMV) infection (gastritis, retinitis, colitis), oral or gastrointestinal candida infection, bacterial pneumonia, and herpes simplex or zoster infection. In one series of 75 patients receiving MMF for reversal or refractory renal allograft rejection (usually combined with maintenance cyclosporin and prednisone), the overall infection rate was 40 %; the most frequent infections were due to CMV (17 %), candida (9%), herpes zoster (4 %), and herpes simplex (3 %). These incidences were considered normal for highly

immunocompromised patients. With relatively high doses of oral MMF for treatment of psoriasis, upper respiratory tract infection symptoms and flu-like symptoms were reported in 21 % and 17 % of patients, respectively. For all of these infections, incidences remained relatively constant during continued therapy (for up to 13 years).

◊ Carcinogenic effects

One of the most disturbing aspects of therapy with oral MMF in earlier studies involving psoriasis patients was its association with malignant neoplasm, including adenocarcinoma of the breast and colon, basal cell carcinoma, carcinoma of the gall bladder, histiocytic lymphoma, glioblastoma multiforme, and squamous cell carcinoma of the epiglottis. In one series, neoplasms were reported in 6 of 76 patients treated with MMF for longer than 1 year; these patients were 52 to 77 years of age at diagnosis, and had received the drug for periods of 3 to 11 years. Although data are inconclusive regarding a cause-effect relationship, the possibility of neoplasm development during prolonged treatment with MPA or MMF should be borne in mind. This is of particular consideration in patients with a history of carcinoma. In limited studies employing MMF in heart or renal transplant patients and rheumatoid arthritis patients, malignant neoplasms have not been reported.

◊ Sepsis

Sepsis has been one of the principal adverse reactions associated with the administration of MMF. Sepsis has occurred in approximately 18 % of renal and cardiac transplant patients in clinical trials.