

Nanomedicines in renal transplant rejection – focus on sirolimus

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Abstract: Nanomedicine, known as the application of nanotechnology in medicine, has been applied to overcome the problems of poor bioavailability, in vitro and in vivo stability, and targeted delivery in the preparation of pharmaceutical products. Sirolimus, a water-insoluble immunosuppressant, has been formulated into an oral solid dosage form by using NanoCrystal[®] technology to increase the water solubility and thereby the bioavailability. The efficacy, safety, and pharmacokinetic properties are not significantly different between liquid and solid formulations except that less fluctuation of sirolimus blood concentration was observed in solid dosage form. The tablet formulation offers the advantages of better palatability and more convenience for long-term use. Sirolimus tablets are not only a successful example of nanomedicine, but also a more cost-effective treatment in renal transplantation than cyclosporine and tacrolimus.

Keywords: nanomedicine, sirolimus, kidney transplantation, transplantation

Introduction to nanomedicine and issues of transplant rejection

It has been more than 51 years since the first successful human kidney transplantation at the Peter Ben Brigham Hospital in Boston (Merrill et al 1956). Graft rejection is one of the greatest challenges to transplantation. It is the outcome of the natural response of the immune system to a foreign substance, or antigen. This complex process is mainly T-lymphocyte mediated, although it involves serial interactions between foreign antigens, T lymphocytes, macrophages, cytokines (also known as lymphokines or interleukins), adhesion molecules (ie, co-stimulatory molecules), and membrane proteins that enhance binding of T lymphocytes and B lymphocytes (Halloran and Miller 1996; Valente and Alexander 1998). The goal of immunosuppressive therapy is to prevent and treat organ rejection as well as prolong graft and patient survival. With the development of immunosuppressive agents (Table 1), kidney transplantation has become one of the most important renal replacement therapies nowadays (Morris 2004).

During the “azathioprine era” from the 1960s to the early 1980s, 1-year cadaveric graft survival rate was around 60% (Morris 2004; Sayegh and Carpenter 2004). With the introduction in 1978 of cyclosporine, a calcineurin inhibitor (CNI), the 1-year graft survival rate increased to more than 80% (Sayegh and Carpenter 2004). In the 1990s, the availability of several other immunosuppressive agents with different mechanisms of action (tacrolimus, mycophenolate mofetil, and sirolimus) made combination immunosuppressive therapy a general modality in organ transplantation. However, due to the potency of these agents and inter- and intra-individual variability in pharmacokinetics, dose individualization is required to maintain adequate immunosuppression while minimizing adverse reactions.

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Table 1 Immunosuppressive agents commonly used in transplantation

| Drugs | Indication in transplantation |
|---|-----------------------------------|
| Protein drugs | |
| Monoclonal antibody | |
| Basiliximab (Simulect [®]) | Induction |
| Daclizumab (Zenapax [®]) | Induction |
| Muromonab-CD3 (Orthoclone OKT3 [®]) | Induction, treatment of rejection |
| Polyclonal antibody | |
| Antithymocyte immunoglobulin rabbit (Thymoglobulin [®]) | Induction, treatment of rejection |
| Antithymocyte immunoglobulin equine (ATGAM [®] , Lymphoglobulin [®]) | Induction, treatment of rejection |
| Corticosteroids | |
| Methylprednisolone | Treatment of rejection |
| Prednisone, prednisolone | Maintenance therapy |
| Immunophilin-binding drugs | |
| Calcineurin inhibitors (CNI) | |
| Cyclosporine (Neoral [®] , Sandimmune [®]) | Maintenance therapy |
| Tacrolimus (Prograf [®]) | Maintenance therapy |
| Mammalian target of rapamycin inhibitors (m-TOR inhibitor) | |
| Sirolimus (Rapamune [®]) | Maintenance therapy |
| Everolimus (Certican [®]) | Maintenance therapy |
| Antiproliferative agents | |
| Antimetabolites | |
| Azathioprine (Imuran [®]) | Maintenance therapy |
| Purine synthesis inhibitors | |
| Mycophenolate mofetil (CellCept [®]) | Maintenance therapy |
| Mycophenolate sodium (Myfortic [®]) | Maintenance therapy |

Poor water solubility and bioavailability contribute to the complexity of dosing cyclosporine and sirolimus. Pharmaceutical companies have been formulating insoluble drugs into commercially available products and minimizing the problem of large intra- and inter-individual variability in pharmacokinetic profiles. A limited number of methods such as generation of ionized drugs, co-solvents, surfactants, soft-gel technology, micronization, and nanotechnology have been utilized in formulating water-insoluble drugs. In addition, other particular drug carriers, such as emulsion, liposome, and polymeric micelle, are considered as alternative methods to deliver water insoluble drugs (Yokoyama 2005). For example, cyclosporine has been emulsified by bile salts to form micelles and further modified into microemulsion from the olive oil-based solution to enhance bioavailability (Thomas et al 2005). The liposomal formulation of tacrolimus also has been demonstrated as an effective strategy to increase efficacy and decrease toxicity (McAlister et al 1999; Alemdar et al 2004). The formulation of amphiphilic block co-polymer micelles has been used to prepare injectable formulations of

the highly lipophilic sirolimus (Ashok et al 2004; Forrest et al 2006).

After the introduction of tacrolimus (a CNI with a potency 32–100 times that of cyclosporine), less variation in bioavailability and not requiring bile salts for oral absorption made tacrolimus a better choice than cyclosporine (Sandimmune[®], Novartis) in liver transplantation (Scott et al 2003). This forced a reformulation of cyclosporine to retain its market share. The new formulation of cyclosporine (Neoral[®], Novartis) is a microemulsion utilizing 9.5% w/v alcohol, corn oil-mono-di-triglyceride, polyoxyl 40 hydrogenated castor oil, and polyethylene glycol as vehicle (PDR 2006). This is an application of microtechnology to drug delivery, a breakthrough in the 1990s.

In the 2000s, a more advanced technology called nanotechnology was applied to medicine, which led to the emergence of nanomedicine (Weber 1999). Nanomedicine is the application of nanotechnology to medicine (Freitas 2005). More broadly, it is the process of applying molecular tools and molecular knowledge of the human body in diagnosis, treatment, and prophylaxis of disease, as well as preserving and improving human health (Freitas 2005). Many nanomedicine technologies have been developed and are close to fruition. Among them, nanostructured materials such as fullerenes have been used in pharmaceuticals (Freitas 2005). Sirolimus (Rapamune[®]; Wyeth-Ayerst, Philadelphia, PA, USA) tablet is another example of the successful application of nanotechnology in pharmaceuticals to overcome the problems of formulation, poor bioavailability, and erratic absorption.

Chemistry, nanocrystal technology used, and formulation of sirolimus

Sirolimus is a triene macrolide antibiotic isolated from *Streptomyces hygroscopicus* (Vezina et al 1975; Sehgal 1998). It is challenging to formulate sirolimus into either an intravenous or oral dosage form due to its water insolubility and a logP (log of the octanol-water partition coefficient, a measure of a drug's lipophilicity) of greater than 5. The solubility of sirolimus is 2.6 µg/mL, which is far below the target solution concentration of 1 mg/mL (Simamora et al 2001). It is impossible to enhance the solubility of sirolimus by the generation of a salt form because of the lack of an ionizable group of sirolimus in the pH range of 1–10 (Simamora et al 2001). The solubility of sirolimus in a single organic solvent, such as ethanol, γ -butyrolactone, dimethyl isosobide, and glycerol formal (a mixture of 5-hydroxy-1,3-dioxane and 4-hydroxymethyl-1,3-dioxolane

in a ratio of 60:40), increases thousands-fold to greater than 90 mg/mL. Although a thousand-fold decrease in the solubility of sirolimus was observed in single cosolvent–water mixtures, the solubility could be increased to more than 10 mg/mL when it was prepared in multiple cosolvent and hydrotrope mixtures, such as 10% ethanol, 40% propylene glycol, 5% benzyl alcohol, and 3%–5% benzoate buffer (Simamora et al 2001). In 1999, the first commercially available product of sirolimus was thus an oral solution with a concentration of 1 mg/mL in Phosal 50 PG (a dispersion of 50% phosphatidylcholine in a propylene glycol/ethanol carrier) and polysorbate 80 (PDR 2003). The taste, and the requirement for refrigerator storage, protection from light, and disposal of the oral syringe after a single use make the oily solution an inconvenient dosage form (Vasquez 2000).

To deliver sirolimus orally in a solid dosage form, the major problem of the dissolution of sirolimus from the dosage form had to be overcome, as well as its permeability across the biological barrier, such as first-pass effect via intestinal and hepatic metabolism and efflux transporter P-glycoprotein (Figure 1). It took one more year to explore the oral tablet formulation of sirolimus using NanoCrystal® technology acquired by Elan Corporation (Rosen and Abribat 2005). This product, launched in 2002, offers greater palatability and convenience of administration and storage (at 20–25°C).

Sirolimus is formulated as nanometer-sized drug crystals by NanoCrystal technology using high-shear media mills. According to the Noyes-Whitney equation of dissolution

(Noyes and Whitney 1897), the rate of dissolution is proportional to the effective surface area (A) and the difference $(C_s - C)/h$:

$$\text{Dissolution rate} = \frac{AD}{h} (C_s - C).$$

Where D is the diffusion coefficient of the drug, h is the thickness of diffusion boundary layer, C_s is the saturation solubility of the drug, and C is the concentration of drug in the medium. Nanocrystalline sirolimus not only increases the effective surface area (A), but also increases the saturation solubility (C_s) and decreases the thickness of the diffusion boundary layer (h) (Muller et al 2001; Merisko-Liversidge et al 2003; Muller and Keck 2004). The saturation solubility can be increased only when the particle size is reduced in a submicron range (Muller and Keck 2004). In addition, it possesses long-term physical stability without Ostwald ripening (the tendency for larger particles to grow in diameter over time, while the smaller particles dissolve in a highly dispersed systems) due to the uniform nanometer-sized particles (Muller and Keck 2004). High adhesiveness on biological surfaces and high endocytosis of nanocrystalline drugs were also reported (Kayser 2000; Muller and Keck 2004). Therefore, nanocrystalline sirolimus may provide a range of improvements in bioavailability, dose proportionality, absorption variability, and absorption rate.

In the process of sirolimus nanocrystallization, generally recognized as safe (GRAS) stabilizers were added to avoid the agglomeration/aggregation of the drug crystals due

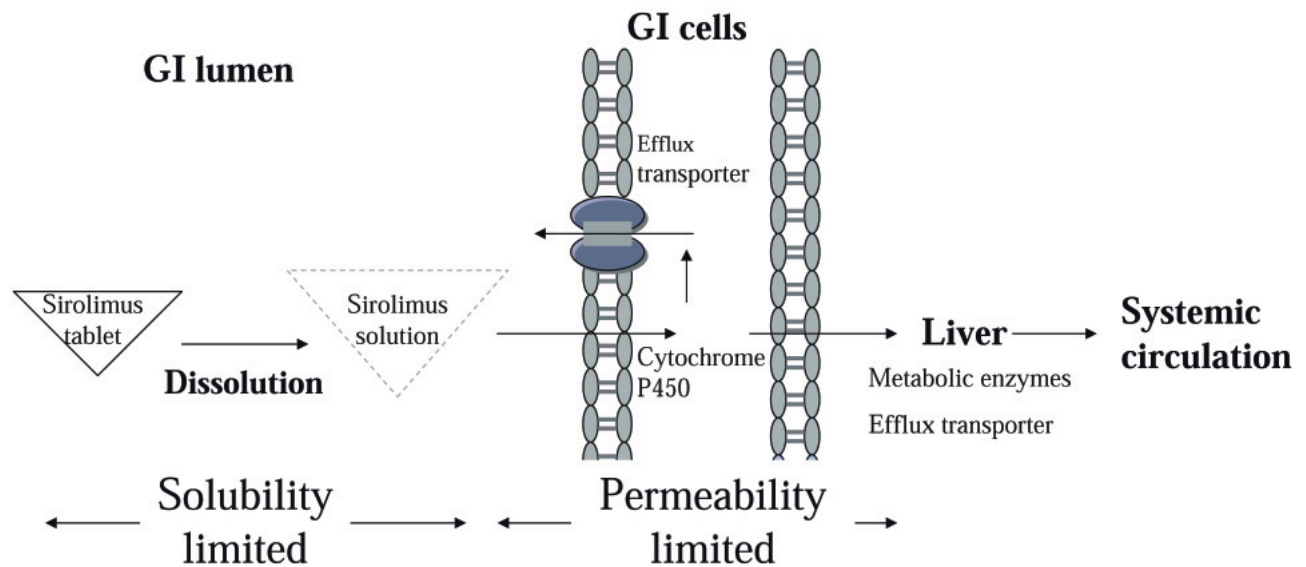


Figure 1 Factors influencing the bioavailability of oral tablet sirolimus. Generally, the bioavailability of oral tablet sirolimus is determined by the solubility and permeability of sirolimus. The nanocrystalline sirolimus improves sirolimus dissolution, saturation solubility, and stability in gastrointestinal (GI) lumen and thereby improves the absorption. However, sirolimus is the substrate for the metabolic enzyme (cytochrome P450 3A) and efflux transporter (P-glycoprotein) in intestinal and hepatic cells. Therefore, less than 20% of sirolimus can reach systemic circulation.

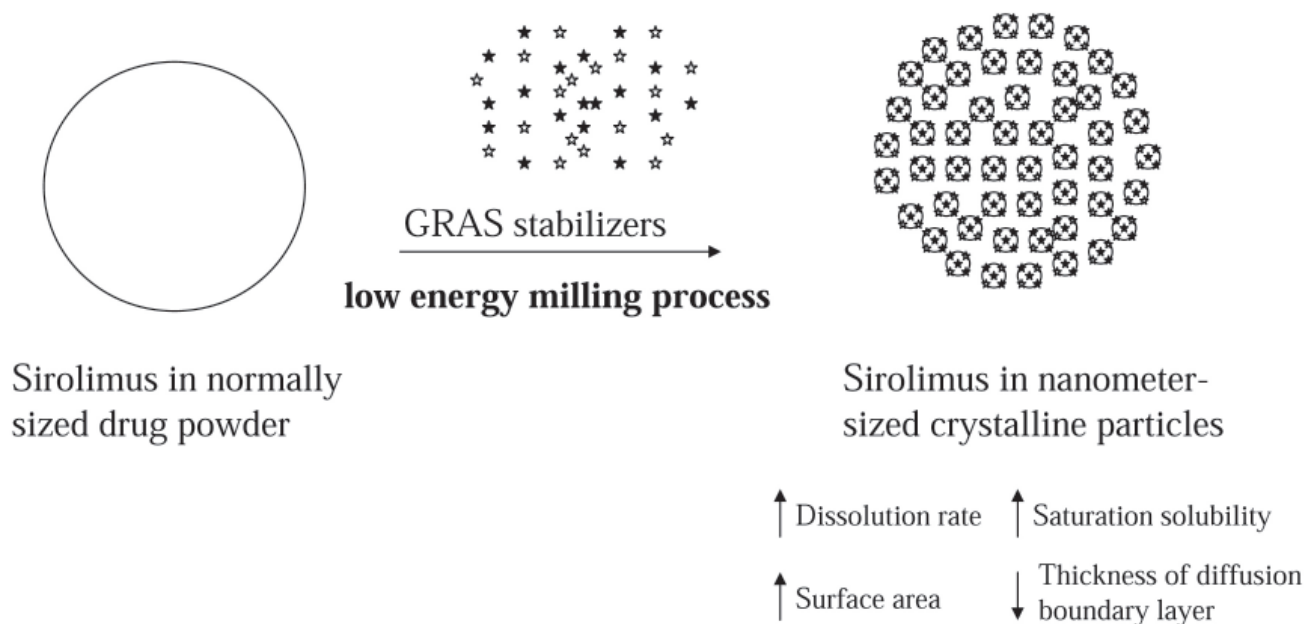


Figure 2 The nanocrystalline sirolimus system. Generally recognized as safe (GRAS) stabilizers were milled with sirolimus into nanocrystal particles by NanoCrystal® technology.

to surface energy of nanocrystalline particles (Figure 2) (Merisko-Liversidge et al 2003). A mixture of non-ionic and ionic stabilizers, such as cellulose, poloxamer, polysorbates, lecithin, sodium glycocholate and polyvinylpyrrolidones, is required to create a steric barrier and an electrostatic repulsion among particles, respectively (Patravale et al 2004). The inactive ingredients of sirolimus tablet include microcrystalline cellulose, polyethylene glycol 8000, polyethylene glycol 20000, poloxamer 188, sucrose, lactose, calcium sulfate, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, glyceryl monooleate, carnauba wax, and other ingredients (PDR 2003). Some of the disclosed and concealed inactive GRAS ingredients were the essential components in the process of sirolimus nanocrystallization.

Immunopharmacology and pharmacokinetics of sirolimus

Sirolimus, with a chemical structure related to tacrolimus, is one of the most potent immunosuppressive agents for prevention of rejection (Sehgal 1998; Vasquez 2000). When combined with cyclosporine- or tacrolimus-based regimen in kidney transplantation, sirolimus increases immunosuppressive activity through a sequential synergistic mechanism.

Like cyclosporine and tacrolimus, sirolimus binds to cytosolic receptors, an intracellular protein, known as immunophilins (Figure 3). Cyclosporine binds to cyclophilin

of the immunophilin family, forming a complex that then engages calcineurin (Clipstone and Crabtree 1992). Both sirolimus and tacrolimus engage another immunophilin called FK-binding protein-12 (FKBP-12). Tacrolimus-FKBP complex, like cyclosporine-cyclophilin complex, inhibits the catalytic activity of calcineurin-calcium-calmodulin complex. The inhibition of calcineurin activity decreases the production of IL-2 and other cytokines, thereby blocking their activation of T-lymphocytes in the G_0 and G_1 phases of cell cycle.

In contrast, sirolimus-FKBP complex binds to the mammalian target of rapamycin (mTOR), thereby inhibiting T-lymphocyte proliferation through inhibition of interleukin-2-mediated (IL-2-mediated) signal transduction in mid to late G_1 phase and preventing cell-cycle progression from the G_1 phase to the S phase (Vathsala et al 1990; Sehgal 1998). It also inhibits IL-2 dependent and independent B-lymphocyte proliferation and antibody production (Aagaard-Tillery and Jelinek 1994).

Sirolimus oral solution has an oral bioavailability of about 15% and distributes widely in tissue (volume of distribution 19 L/kg; Yatscoff 1996; Mahalati and Kahan 2001). The mean oral bioavailability of sirolimus tablets is not significantly different from that of oral solution (Kelly et al 1999; Hariharan and Zimmerman 2000; Kelly et al 2000; PDR 2003). The tablets are not bioequivalent to the oral solution. However, clinical equivalence has been demonstrated at the 2-mg dose level (VanBuren 2000; PDR 2003). At doses

between 3 and 12 mg/m², there is a linear correlation between the whole-blood concentration and dosage of sirolimus solution within the same transplant individuals (PDR 2003). The mean time-to-peak concentration (t_{max}) is about 1 hour after a single dose in healthy volunteers and 2 hours after multiple oral doses in renal transplant recipients (PDR 2003). A study by Kelly et al (1999) indicated that there were no differences between the liquid and tablet in trough concentrations (C_0) and area under the concentration–time curve (AUC) during dosage form conversion in stable kidney transplant recipients. However, tablets had a lower whole-blood peak concentration (C_{max}) and dose corrected C_{max} . The t_{max} of tablets was longer, but was significant only at 4 weeks post-conversion. The inter-individual variability and percentage fluctuation of sirolimus serum concentration were less in tablets than in liquids (Kelly et al 2000). These results indicate that sirolimus tablets provide more consistent immunosuppressive exposure over the dosing interval in each individual than the liquid formulation.

Sirolimus is metabolized extensively by liver enzymes, with only 2.2% excreted unchanged in urine (PDR 2003). A study by Wu et al (2005) found that the dose-adjusted trough concentration of sirolimus in patients with persistent liver enzyme elevation was significantly higher than in those with normal liver function. Like cyclosporine and tacrolimus, sirolimus is a substrate for both cytochrome P450 (CYP) 3A and P-glycoprotein (P-gp) (Sattler et al 1992; Turgeon et al 1994; Lampen et al 1995; Fricker et al 1996; Yatscoff 1996; Lown et al 1997; Masuda et al 2000), and thus has potential

drug interactions similar to those of CNIs. In addition, the potential drug interactions between CNIs and sirolimus have been a concern. Sirolimus did not significantly affect the pharmacokinetics of either an olive oil-based or the microemulsion-formulated cyclosporine in human studies (Zimmerman and Kahan 1997; Kahan et al 1998; MacDonald et al 2000). A limited number of available studies did not reveal an effect of tacrolimus on the pharmacokinetics of sirolimus (MacDonald et al 2000; McAlister et al 2002; Kuypers et al 2003; Undre 2003). However, both an olive oil-based or the microemulsion-formulated cyclosporine may increase the bioavailability of sirolimus (PDR 2003). In addition, the bioavailability and trough concentrations of sirolimus are significantly higher when microemulsion-formulated cyclosporine and sirolimus are administered concomitantly than when they are staggered by 4 hours (Kaplan et al 1998; MacDonald et al 2000; PDR 2003). Thus, the manufacturer of sirolimus, Wyeth Ayerst (Philadelphia, PA), has suggested that the two drugs be given 4 hours apart. However, a randomized, parallel-group study has shown that during multiple-dose administration, the bioavailability and trough concentration of sirolimus were, respectively, 1.46 and 1.42 times higher in the cyclosporine group than in the tacrolimus group, even though sirolimus was administered 6 hours after CNIs (Wu et al 2005). Staggered administration cannot completely prevent a drug interaction between cyclosporine and sirolimus solution. Whether sirolimus tablets exert the same drug interaction with cyclosporine remains to be studied.

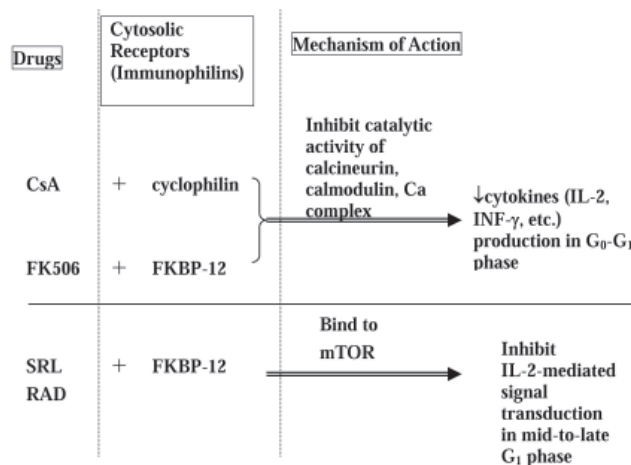


Figure 3 Mechanism of action of cyclosporine, tacrolimus, and sirolimus. Adapted with permission from Wu FL, Tsai MK, et al. 2005. Effects of conversion from sirolimus oral solution to tablets in stable Taiwanese renal transplant recipients. *J Formos Med Assoc*, 104:22-8. © The Formosan Medical Association.

Abbreviations: CsA, cyclosporine; Ca, calcium; FK506, tacrolimus; KBP, FK binding protein; INF- γ , γ -interferon; IL, interleukin; mTOR; mammalian target of rapamycin; RAD; everolimus; SRL, sirolimus.

Efficacy, comparative studies, and prolonged therapy

In September 1999, sirolimus was approved by the US Food and Drug Administration (FDA) for use in combination with cyclosporine and steroids. In November 2000, the drug was registered by the European Agency as an alternative to CNI for maintenance therapy. In April 2003, the FDA approved a new regimen to reduce the use of cyclosporine by substituting higher doses of sirolimus (Kahan 2004).

The safety and efficacy of sirolimus oral solution and tablets for the prevention of graft rejection following kidney transplantation has been compared in a 477-patient, randomized, multicenter, controlled trial. There was no significant difference between the oral solution and tablet formulation for rejection, graft, and patient survival at 3, 6, and 12 months after transplantation (VanBuren 2000; PDR 2003).

A CNI-free sirolimus-based protocol may avoid CNI

nephrotoxicity. The incidences of acute rejection, chronic graft failure, and graft loss associated with this sirolimus-based therapy were 19%, 14%, and 10%, respectively (Kahan 2004). The long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal have been demonstrated by Kreis et al (2004). Lipid profiles were similar between groups, while blood pressures and incidence of abnormal kidney function, edema, hyperuricemia, hyperkalemia, gingival hyperplasia, and *Herpes zoster* were significantly lower in the sirolimus-based therapy. Recipients with low to moderate rejection risk may consider stopping cyclosporine 2–4 months after transplantation (Halloran 2004; Kreis et al 2004). Compared with other immunosuppressive agents, regimens containing sirolimus have a lower incidence of cytomegalovirus (CMV) and BK polyoma virus infection (Halloran 2004; Kahan 2004).

Different from other immunosuppressive agents, m-TOR inhibitors may have antineoplastic and arterial protective effects. Sirolimus slows the growth of established experimental tumors (Guba et al 2002). According to Kahan's study group (Kahan 2004), sirolimus therapy outweighs other immunosuppressive agents in the low incidence of malignancy. Compared with the general US population, the sirolimus/cyclosporine therapy showed equal incidence of skin cancers, and a 4-fold increase in post-transplant lymphoproliferative disorder (PTLD) and renal cell carcinoma. It was also reported to inhibit the progression of dermal Kaposi's sarcoma in kidney-transplant recipients while providing effective immunosuppression (Stallone et al 2005). The incidence of Kaposi's sarcoma among solid organ recipients is about 500 times that in the general population (Hayward 2003). Three months after switching from cyclosporine to sirolimus in 15 kidney-transplant recipients who had biopsy-proven Kaposi's sarcoma, all cutaneous Kaposi's sarcoma lesions disappeared in all patients (Guba et al 2002).

Incorporation of sirolimus into coronary stents inhibits restenosis (Morice et al 2002; Dibra et al 2005). Combination of everolimus with CNIs reduced the incidence of graft coronary artery disease associated with heart transplantation (Eisen et al 2003). However, the potential arterial protective effects of m-TOR inhibitors must be weighed against their hyperlipidemia side-effect (Blum 2002).

Tolerability

The major adverse reactions of sirolimus include hyperlipidemia, thrombocytopenia, and impaired wound healing (PDR 2003). Delayed recovery from acute tubular

necrosis in kidney transplants, aggravation of proteinuria, mouth ulcers, pneumonitis, and skin lesions were also reported (Halloran 2004). Theoretically, sirolimus should not cause nephrotoxicity due to lack of CNI activity (Sehgal 1998). Its combination with CNIs was expected to decrease nephrotoxicity of CNIs due to dose reduction of CNIs (McAlister et al 2000; Kahan and Camardo 2001). However, the combinations have been reported to increase nephrotoxicity, the hemolytic-uremic syndrome, and hypertension (Gonwa et al 2003; Halloran 2004). It should be noted that the dose and whole-blood concentrations of CNIs and sirolimus should be taken into consideration in interpreting these data. For example, in the study by Gonwa et al (2003), there was no difference between the two groups in median tacrolimus whole-blood trough concentration (8.5 ng/mL and 8.7 ng/mL in the sirolimus- and mycophenolate-treatment groups, respectively). That is, there was no dose reduction of tacrolimus in the sirolimus-treatment group. The median sirolimus dose was 3 mg/day with a corresponding median whole-blood trough concentration 7.3 ng/mL at 6 months, which was higher than 2 mg/day (the dosage usually recommended). Dose reduction of CNIs as well as minimizing the dose of sirolimus is crucial to minimize toxicity of this combination. Withdrawing one of the drugs in the combination of a m-TOR inhibitor and cyclosporine can also reduce renal dysfunction and hypertension, with a small increase in rejection episodes (Johnson et al 2001).

Pharmacoeconomic data and patient acceptability

With the help of the nanotechnology, tablet formulation of sirolimus was successfully introduced onto the market one year after the launch of the oil-based liquid formulation. Patient satisfaction is greater with sirolimus tablet than oil-based liquid form because of improvement in palatability, less restricted storage conditions, and greater convenience of administration. Tablet sirolimus is now the preferred dosage form except in those who require a lower dose or cannot take tablets.

Sirolimus has been recognized as a potent immunosuppressive agent with less nephrotoxicity than CNIs, such as tacrolimus and cyclosporine. The cost-effectiveness and cost-utility studies of sirolimus versus tacrolimus and cyclosporine in kidney transplant patients have been reported by McEwan et al (2005, 2006). A stochastic simulation model was developed using clinical trial and observational data to forecast the incidence of graft failures, hemodialysis, peritoneal dialysis, retransplants, and acute rejections. Using

historical data on 937 renal transplant recipients, the costs of the different events from the perspective of the UK National Health Service, valued at 2003 prices with discount, were then used to evaluate both cost-effectiveness and cost-utility over 10 and 20 years after transplantation (McEwan et al 2005, 2006). Treatment with sirolimus was projected to gain 0.72 and 1.8 discounted years of functioning graft over tacrolimus over 10-year and 20-year time horizons, respectively (McEwan et al 2005). Similarly, when compared with cyclosporine, sirolimus gained 0.6 and 1.59 discounted years of functioning graft and a cost saving of US\$486 and US\$13033 per patient over 10-year and 20-year time horizons, respectively (McEwan et al 2006).

Conclusions

Sirolimus is one of the most potent immunosuppressive agents in transplantation which is useful in combination with CNI or as an alternative to CNI for maintenance therapy. Although sirolimus is correlated with higher incidence of hyperlipidemia, thrombocytopenia, impaired wound healing, delayed graft function, proteinuria, mouth ulcers, pneumonitis, and skin lesions, it has antineoplastic and arterial protective effects, and is associated with lower incidence of CMV infection in transplant recipients. With the application of nanotechnology, NanoCrystal formulation overcomes the problems of formulation, poor bioavailability, and erratic absorption of sirolimus. The tablet formulation has a better palatability, and is more convenient for long-term use. In addition, cost-effectiveness and cost-utility analysis also demonstrated the benefits of long-term use of sirolimus in kidney transplantation. Using nanocrystalline sirolimus as a successful prototype, nanotechnology is a prominent approach in the formulation of water-insoluble drugs for clinically applicable dosage forms in the future.

Disclosures

Neither of the authors has any conflict of interest.

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