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Review Article

Recent Developments in Drug Delivery to Prolong Allograft Survival in Lung Transplant Patients

Alan B. Watts and Robert O. Williams III
Division of Pharmaceutics, College of Pharmacy, University of Texas at Austin, Austin, TX, USA

Jay I. Peters
Division of Pulmonary Diseases/Critical Care Medicine, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Since the discovery of cyclosporine in 1971, calcineurin inhibitors have played a critical role in the therapeutic suppression of the immune response. Patients receiving solid organ transplants rely heavily on these medications to prevent the acute and chronic rejection of allografted tissue. These therapies can prove difficult because of potential toxicity, heightened risk of invasive infection, and erratic oral bioavailability, requiring frequent blood samples for monitoring of systemic levels. Added challenges are presented in immunosuppression of lung transplant patients owing to the increased susceptibility to invasive infection and extensive immune mechanisms inherent in lung tissue. With the introduction of tacrolimus, a more potent calcineurin inhibitor, clinical outcomes of transplants have continued to improve; however, little improvement has been noted in lung transplantation. While very effective upon arrival at the site of action, tacrolimus and cyclosporine present a variety of formulation challenges such as poor solubility, potential systemic toxicity, and extensive first pass metabolism. Initial attempts to improve solubility in both oral and intravenous formulations have resulted in variable drug absorption and increased systemic toxicity, respectfully, creating a need for formulation improvement. Through alternative routes of delivery and novel formulation techniques, researchers have addressed these issues and, in some cases, demonstrated improved clinical outcomes. Through enhanced solubilization, reduction in absorption variability, and more effective drug targeting with reduced systemic levels, improvements in outcomes and overall patient survival in lung and other solid organ transplantation can be expected.

Keywords immunosuppressant; lung transplant; calcineurin inhibitor; cyclosporine; tacrolimus; poorly water-soluble drug

Address correspondence to Robert O. Williams III, Division of Pharmaceutics, College of Pharmacy (Mailstop A1920), University of Texas at Austin, Austin, TX 78712-1047, USA. E-mail: williro@mail.utexas.edu

INTRODUCTION

Of the major forms of solid organ transplantation (kidney, liver, heart, and lung), lung transplantation has proven to be the most difficult in providing effective immunosuppression while maintaining the body's ability to protect against invasive infection. Further risks of infection are added in lung-transplanted patients by the disruption of the mucociliary escalator and cough reflex (Egan, 2004). While consistently improving, survival of lung transplant patients lags behind other forms of transplantation with an approximate 50% survival rate after 5 years. Chronic allograft rejection caused by bronchiolitis obliterans syndrome (BOS) affects 50–60% of 5-year postoperative patients and is a major hurdle in improving quality of life and patient survival (Estenne & Hertz, 2002). To prevent chronic rejection, maintenance immunosuppressive therapies consisting of a three-drug regimen (calcineurin inhibitor, antimetabolite, and corticosteroid) are typically administered (Knoop, Haverich, & Fisher, 2004). Calcineurin inhibitors hinder T-cell proliferation, and the subsequent immune response, by complexing with immunophilins and binding to calcineurin, resulting in the inhibition of cytokine transcription. Antimetabolites, such as azathioprine and mycophenolic acid, reduce expansion of T and B cells by blocking purine synthesis (Mueller, 2004). Corticosteroids have a broad mechanism of action which is not completely understood (Lee, 2003); however, they are effective inhibitors of T-cell and macrophage cytokine production. Withdrawal from prolonged corticosteroid therapy can be particularly troublesome in lung transplant patients due to dangerous side-effects including hypertension, increased cholesterol, and osteoporosis (Borro, Sole, De la Torre, Pastor, & Tarazona, 2005; Meier-Kriesche et al., 2006). A new method of therapy has been developed in polyclonal and monoclonal antibodies. Monoclonal antibodies such as basiliximab and daclizumab bind specifically to interleukin-2R (IL-2R) surface protein, inhibiting signaling for further T-cell expansion. A schematic depicting the cycle of

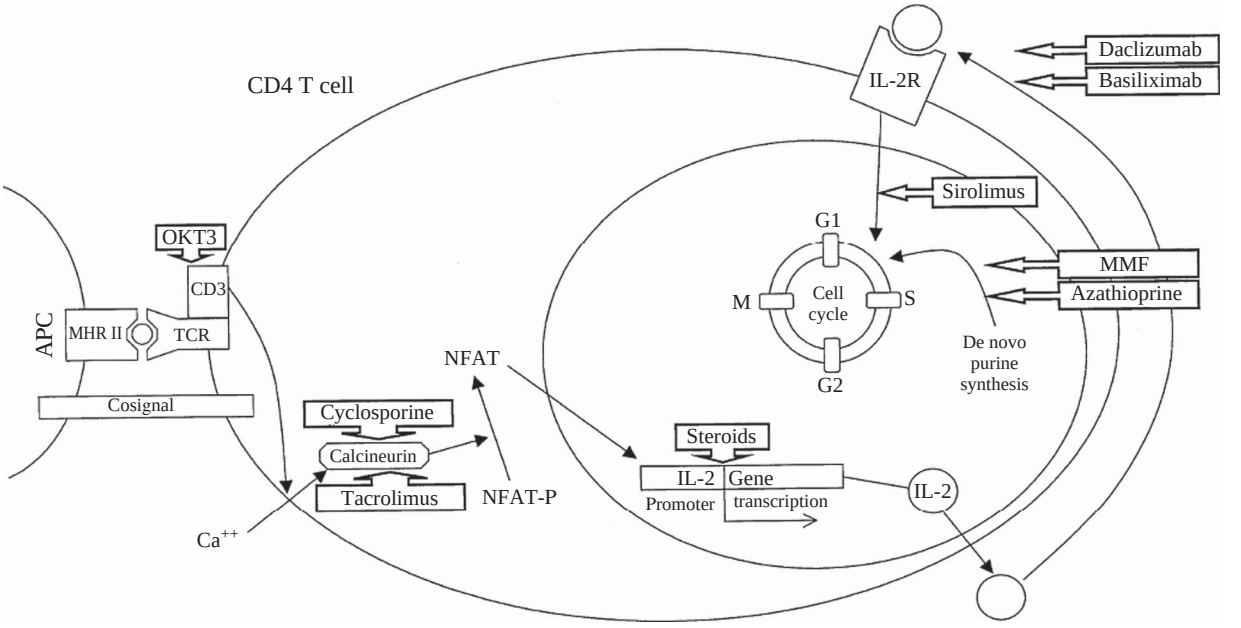

FIGURE 1. Cycle of CD4 T-cell activation with sites of immunosuppressive action. (Reprinted with permission from Mueller, 2004.)

CD4 T-cell activation with sites of immunosuppressive action is shown in Figure 1. Development of new immunosuppressive drugs (i.e., daclizumab, basiliximab, everolimus, and FK788) has further raised expectations of successful immunosuppressive therapy. The purpose of this review article is to summarize how advances in drug delivery may lead to improved clinical outcomes specifically relating to lung transplants. The majority of research done in this area investigates improved delivery of calcineurin inhibitors cyclosporine, and tacrolimus.

In this review, innovative drug delivery with the potential to improve survival rate in lung-transplanted patients is discussed. Many of these delivery methods are currently used extensively in transplantation therapy and also may be applied to lung transplant recipients while other novel methods are specifically intended for rejection prevention specifically in transplanted lung patients.

DELIVERY SYSTEMS INTENDED FOR THE ORAL ROUTE OF ADMINISTRATION

Consistent oral immunosuppressive regimens are common in the clinical setting as well as during long-term maintenance therapies. Self-administration and patient compliance make the oral delivery route attractive; however, toxic side-effects, variations in clearance (Antignac et al., 2005), and bioavailability of some poorly soluble immunosuppressive drugs present obstacles in maintaining therapeutic drug levels. Many novel formulation approaches have been developed for lung (Carby & Lyster, 2006; Mulligan & Wood, 2003), as well as other solid organ transplant recipients, to prevent acute and chronic allograft rejection.

Cyclosporine

In all solid organ transplantation, including lung transplantation, oral cyclosporine has been frequently used to block T-cell proliferation although, due to its improved potency, oral tacrolimus (Prograf®, Astellas Pharma Inc., Tokyo, Japan) has replaced cyclosporine in many transplant centers (Meier-Kriesche et al., 2006) (Figure 2). Cyclosporine, like many other lipophilic drugs, suffers from low bioavailability and has been extensively studied for formulation methods to improve drug delivery. The Biopharmaceutics Classification System (BCS) Guidance classifies cyclosporine as a class IV drug, meaning that it is both poorly water soluble and poorly permeable. Despite a high affinity for hydrophobic molecules (log P = 2.92), cyclosporine remains poorly preamble because of its high molecular weight and rigid cyclical structure (Italia, Bhardwaj, &

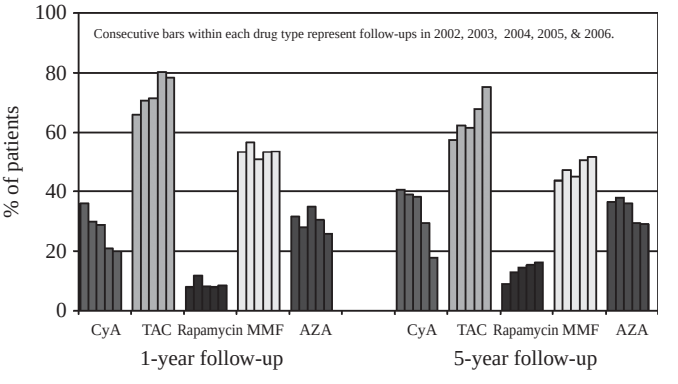

FIGURE 2. Maintenance of immunosuppressive drug use in adult lung recipients: AZA, azathioprine; CyA, cyclosporine; MMF, mycophenolate mofetil; TAC, tacrolimus. (Reprinted with permission from Trulock et al., 2007.)

Ravi Kumar, 2006). Oral delivery of this drug is further hindered by *p*-glycoprotein-mediated efflux and extensive degradation by cytochrome P450 during first pass metabolism. Variability in bioavailability of orally administered cyclosporine in immunosuppressed patients has been shown to be more influenced by *p*-glycoprotein levels rather than by intestinal enzymes (Hebert, 1997). It has been suggested by some that limiting the role of *p*-glycoprotein action is an effective way to reduce variability in oral immunosuppressant absorption, consequently reducing incidence of acute graft rejection (Floren et al., 1997; Lo & Burckart, 1999). While metabolic rates may be affected by other medications (i.e., triazole antifungals, macrolide antibiotics, calcium channel blockers), the majority of research has focused on improvement of oral bioavailability through solubility enhancement rather than chemically limiting the body's ability to metabolize these drugs.

The originally marketed oral cyclosporine product, Sandimmun® (Novartis), is available in soft gelatin capsules or as an oral solution dissolved in an ethanol/maize oil formulation. Bioavailability and resulting systemic concentrations of cyclosporine after oral dosing of this oil-based formulation was low because of its dependence on bile salt concentration in the upper small intestine (Metha et al., 1988). Bile salts are needed to elicit micellular solubilization of these oils, and because these salts may vary in concentration on intersubject and intrasubject basis, the overall bioavailability of this product proved to be variable. An improved oral formulation was reported where in situ micoremulsification of cyclosporine occurs as soon as the formulation comes in contact with an external aqueous phase. By utilization of an emulsion stabilizing surfactant (DL-α-tocopherol) (Holt & Johnston, 1997), Neoral® (Norvartis) is able to achieve therapeutic immunosuppressant levels with less variability. In a multicenter, double blind clinical study, efficacy in prevention of episodes of heart transplant rejection was shown to be superior in micoremulsified cyclosporine when compared to the older formulation. As would be expected, reduced variability in blood pharmacokinetic profiles using Neoral® was seen in the first year of treatment (Eisen et al., 2001). Additionally, therapeutic blood targets were met with lower dosing of the microemuslion formulation, proving improved bioavailability. These results agreed with earlier findings reported by Tan et al. in a single-dose study in patients awaiting lung transplant suffering from cystic fibrosis. Overall bioavailability of microemulsified cyclosporine was shown to be 1.84–2.09 times higher (at 200 and 800 mg dose, respectively) than that of the conventional formulation in these patients (Tan, Trull, Uttridge, & Wallwork, 1995).

Other novel oral cyclosporine drug delivery systems have been reported, many of which have not undergone substantial animal and human testing for efficacy and safety in lung transplantation. Engineered particles designed for delivery to lymphatic tissue have been reported by several groups to produce an enhanced effect on T lymphocytes. This strategy demonstrated moderate success in in vivo testing of engineered nanoparticles (El-Shabouri, 2002; Varela et al., 2001). Specifically, El-Shabouri has shown increased absorption through the intestinal mucosa by incorporation of charge carrying polymers which resulted in an increase in overall bioavailability of 73% when compared to microemulsified cyclosporine composition. Improvements in solubility and Caco-2 cell permeability have also been reported by incorporation of synthetic amphiphilic polymers, resulting in micellization of cyclosporine (Francis, Cristea, Yang, & Winnik, 2005). Nanoparticle drug release was effectively targeted to the upper small intestine by incorporation of poly(methacrylic acid and methacrylate) copolymer, and this showed an approximately 15% increase in bioavailability relative to Neoral® (Dai et al., 2004). Other attempts to control release and enhance cyclosporine solubility have incorporated the use of sodium lauryl sulfate-dextrin (Lee, Lee, Choi, & Kim, 2001) and poly(lactic acid)-poly(ethylene glycol) (Gref et al., 2001) microspheres. Enhanced solubilities and rapid dissolution have been shown in poorly soluble drugs when amorphous particles are produced. Processes for making stabilized amorphous cyclosporine have been developed, including evaporative precipitation into aqueous solution (EPAS) (Chen, Young, Sarkari, Williams III, & Johnston, 2002; Hu, Johnston, & Williams III, 2004). Compositions containing polyoxyethylene (40) stearate dispersions (Liu et al., 2006) have also been reported to achieve comparable bioavailability to Neoral® using a solvent-melt technique to produce a stable amorphous powder. Although generally unproven in complex biological models, these formulations should be considered when addressing the future of drug delivery systems developed for improving solid organ transplant rejection rates.

Tacrolimus

Orally administered tacrolimus is the cornerstone of immunosuppressive therapies for all forms of solid organ transplant. In the past 10 years, trends have shown oral tacrolimus use increasing as a maintenance therapy. In 2006, approximately 78% of lung transplant patients 1 year postoperation and approximately 73% of patients 5 years postoperation were using tacrolimus along with steroids and antimetabolites to prevent lung graft rejection (Trulock et al., 2007). The replacement of cyclosporine as a calcineurin inhibition agent with tacrolimus is based on data collected in both the laboratory and the clinical setting. Specifically, patients receiving lung transplants over the course of a 3-year study were given immunosuppressive therapy with either orally administered cyclosporine or tacrolimus. In the first 2 years, survival rates were similar; however, early signs of rejection and cases of obliterative bronchiolitis were seen much more frequently in cyclosporine-dosed patients (38 vs. 21.7%) (Keenan et al., 1995). In a trial in the United Kingdom involving patients having received liver transplants, clinical outcomes were compared between patients dosed with tacrolimus (Prograf®)

and microemulsified cyclosporine (Neoral®). Tacrolimus therapy was initiated at 0.1 mg/kg/day orally and adjusted appropriately to maintain blood trough levels of 5–15 ng/mL, while cyclosporine was given orally at 10 mg/kg/day for maintenance of therapeutic trough levels between 150 and 250 ng/mL. The reduced mortality rate and decreased need for retransplantation shown in this study after 1 year demonstrated the clinical advantage provided by tacrolimus as compared to cyclosporine (O'Grady, Burroughs, Hardy, Elboume, & Truesdale, 2002). Additionally, replacing oral cyclosporine with oral tacrolimus has been shown to improve pulmonary function in lung-transplanted patients as determined by improvements in forced vital capacity and reduced exhaled nitric oxide levels (Verleden, Dupont, Van Raemdonck, & Vanhaecke, 2003). From a pharmacodynamic view point, these two drugs show almost exactly the same method of T-cell inhibition although improvements in therapeutic outcomes using tacrolimus may also be due to a potency that is 10–100 times greater than that of cyclosporine (Jain & Fung, 1996; Keenan et al., 1995). In addition to showing clinical improvements in daily maintenance therapy, oral tacrolimus has been shown to be effective as a rescue therapy in patients experiencing acute rejection while receiving oral cyclosporine (Garrity & Mehra, 2004).

The innovator product, Prograf®, consists of a fine dispersion of amorphous tacrolimus in a hydroxypropylmethylcellulose (HPMC) carrier at 1:1 ratio and is intended for a twice daily dosing regimen. Superior drug solubility was reported for the Prograf® formulation when compared to bulk, crystalline tacrolimus in dissolution testing due to improved tacrolimus solubility (Yamashita et al., 2003). Stabilized amorphous powders are often used in oral formulations as a strategy to enhance drug solubility (Vaughn, McConville, Crisp, Johnston, & Williams III, 2006). Overhoff et al. successfully stabilized amorphous tacrolimus with a polymeric carrier by a rapid freezing technique and showed the ability to supersaturate dissolution media in in vitro testing. Further testing of oral dosing in a rat model showed greater systemic absorption when poloxamer 407-stabilized tacrolimus was dosed in comparison to Prograf® (Overhoff et al., 2008). In the granulated formulation of Prograf® intended for oral administration, lactose, croscarmellose sodium, and magnesium state are also included in capsules with the HPMC:tacrolimus combination. In the interest of enhancing patient compliance and reducing medication confusion for transplant recipients, a once-daily tacrolimus formulation, Prograf® XL (formerly MR tacrolimus), has been developed. This once-daily (qd) formulation substantially reduced the maximum concentration (C_{max}) of the tacrolimus in comparison to Prograf® by providing a more gradual drug release while showing a similar extent of absorption (ratio of Prograf® XL to Prograf® for AUC_{0-inf} = 97.3%) (First & Fitzsimmons, 2004). Milligram-for-milligram conversion from twice-daily tacrolimus to qd tacrolimus in clinical studies has been shown to produce equivalent systemic levels in kidney (Alloway et al., 2005; Silva et al., 2007) and liver (Florman et al., 2005) transplant patients in combination with other immunosuppressive therapies. Prograf® XL is associated with improved patient compliance due to reduced dosing frequency; however, variable gastrointestinal absorption with this formulation (also seen with the proprietary product, Prograf® and oral cyclosporin) necessitates close monitoring of trough blood levels.

Although much of the metabolic pathway is still not fully elucidated, tacrolimus is thought to undergo the same metabolic degradation as cyclosporine (Hebert, 1997). When investigating tacrolimus absorption in the rat small intestine, it was found that the presence of *p*-glycoprotein had an inhibitory effect on drug uptake, while the area of the intestine with a lower *p*-glycoprotein concentration, the jejunum, showed the highest absorption rates (Tamura, Ohike, Ibuki, Amidon, & Yamashita, 2002). Somewhat conflicting results were recently reported by Saitoh and coworkers. They found that tacrolimus is neither a substrate nor an inhibitor of *p*-glycoprotein and that cytochrome P450 was responsible for the drug's extensive metabolism (Saitoh et al., 2006). Unlike cyclosporine, tacrolimus is classified as a BCS class II drug, being poorly water soluble (4–12 μg/mL) (Tamura et al., 2002) but highly permeable (log P = 3.3). Currently, some researchers are investigating other methods for enhancement of tacrolimus bioavailability, and many of the drug delivery technologies described for cyclosporine could also be applied for tacrolimus. Arima and coworkers have used various hydrophilic β-cyclodextrins to enhance solubility in in vitro as well as in a rat model, concluding that cyclodextrin solubilized tacrolimus showed a 1.2-fold increase in AUC compared to that of Prograf® (Arima et al., 2001). In a subsequent study, it has also been hypothesized that increased bioavailability of β-cyclodextrin-incorporated tacrolimus in a rat model is due to inhibition of *p*-glycoprotein efflux as well as increased solubilization. Drug-loaded poly(lactide-co-glycolide) (PLGA) biodegradable nanoparticles and pH-sensitive nanoparticles have also been investigated for colonic delivery in the treatment of inflammatory bowel disease (Lamprecht, Yamamoto, Takeuchi, & Kawashima, 2005; Meissner, Pellequer, & Lamprecht, 2006). These formulations also show potential for enhancement of systemic bioavailability in transplanted patients by targeting areas of the gastrointestinal tract where the drug is metabolized to a lesser extent.

Other Agents

Many other oral immunosuppressive therapies such as corticosteroids and antimetabolites are used in the prevention of lung graft rejection (i.e., azathioprine and prednisolone) and are not confronted with the bioavailability obstacles seen in the calcineurin inhibitors discussed above. These drugs, however, are limited in their clinical use and are often used in conjunction with other immunosuppressive agents because of their adverse side effects and narrow therapeutic windows (Lee, 2003).

Some of these drugs do require special considerations when dosing, including mycophenolic acid and sirolimus. Mycophenolate mofetil, a prodrug commonly used as an antiproliferative agent, has been shown to cause irritation in the upper gastrointestinal tract when delivered orally due to the mofetil ester entity. Enteric-coated mycophenolate sodium (Myfrotic®) is offered as an alternative that allows for drug targeting of the upper small intestine, avoiding gastric and upper intestinal irritation. Mycophenolate sodium has been shown to be effective in conjunction with other immunosuppressive regimens in prevention of solid organ graft rejection (Curran & Keating, 2005). Sirolimus, another immunosuppressive agent that blocks interleukin-2 (IL-2) proliferation, has been shown to be effective in transplant patients while avoiding nephrotoxicity normally seen with calcineurin inhibitors. Oral sirolimus (also known as rapamycin) has been shown to have a synergistic effect when dosed with cyclosporine or tacrolimus. As both sirolimus and calcineurin inhibitors are metabolized by cytochrome P450, increases in sirolimus bioavailability may be seen when coadministered because of reduced metabolism (Hebert, 1997). This interaction should be noted by the clinician with doses adjusted accordingly. Systemic toxicity (i.e., bone marrow suppression) and interstitial pneumonitis associated with oral dosing of sirolimus have limited the widespread use of this agent in lung transplant patients (McWilliams, Levvey, Russell, Milne, & Snell, 2003; Pham et al., 2004; Virmani et al., 2004). Additional pulmonary complications associated with oral dosing of sirolimus such as lymphocytic alveolitis and alveolar hemorrhage have been reported to improve upon discontinuation or reduction of dose.

DELIVERY SYSTEMS INTENDED FOR THE PARENTERAL ROUTE OF ADMINISTRATION

Many immunosuppressive therapies are administered intravenously during patient hospitalization, especially in the case of induction therapy. Induction therapy is not advocated by all lung transplant centers, in fact it was preformed in less that 50% of lung transplant cases in recent years (Trulock et al., 2007) (Figure 3). Induction therapy occurs postoperatively and involves dosing of powerful antilymphocyte antibody parenterals to achieve an initial level of immunosuppression. Critics of induction therapy site that "over suppression" might increase risk of infection, whereas advocates of this therapy believe that it helps prevent acute rejection in the critical first few weeks (Brock et al., 2001). Intravenous immunosuppressants such as IL-2R antagonist (basilizimab and daclizumab) and polyclonal antibodies (antithymocyte globulin) are typically used in this therapy. As of yet, no clinical studies have reported data making a strong case for induction therapy in lung transplantation (Uber & Mehra, 2007).

In cases of maintenance therapy, many marketed intravenous formulations are adaptations of the active used in oral

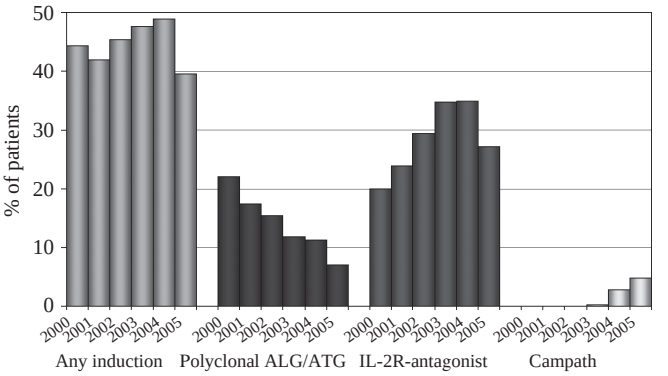

FIGURE 3. Induction therapy by year in adult lung recipients: ALG, antilymphocyte globulin; ATG, antithymocyte globulin; IL-2R, interleukin-2 receptor. (Reprinted with permission from Trulock et al., 2007.)

compositions (as is the case for Prograf® and Neoral®). In Prograf®, polyoxyl 60 castor oil and dehydrated alcohol are used to solubilize tacrolimus. Sandimmune® for injection consists of cyclosporine in Cremophor® EL (castor oil) and alcohol solution. Because of toxicity and high risk of anaphylaxis associated with intravenous castor oil, these formulations are typically avoided in favor of oral immunosuppression (Takamatsu et al., 2001). Other formulations have been studied for prolonged drug release and reduced systemic toxicity through drug targeting. Injected liposomal cyclosporine (Arulsudar, Subramanian, Mishra, Sharma, & Murthy, 2003) and tacrolimus (Moffatt, McAlister, Calne, & Metcalfe, 1999) have been investigated in mice for their effect on therapeutic index and tissue targeting. Evidence of tissue targeting as well as prolonged systemic half lives was observed in both studies due to manipulation of fatty acid chain length, surface charge, and particle size. Novel polymeric micelles of cyclosporine have also been studied and exhibited superior solubility, prolonged drug release, and lowered toxicity when compared with Cremophor® EL (Sandimmune®) solubilized injections (Aliabadi, Mahmud, Sharifabadi, & Lavasanifar, 2005; Guo, Wu, Ping, Chen, & Shen, 2005). Specifically, poly(ethylene oxide)-*b*-poly(ε-caprolactone) (PEO-*b*-PCL) micelles increased solubility and prolonged release of cyclosporine due to high encapsulation efficiencies and reduced molecular mobility within the micelle. Other methods of solubilizers such as ethanol and Cremophor® EL allow for higher molecular mobility and thus faster drug diffusion. In previous studies, it was shown that cyclosporine for injection solubilized with Cremophor® EL causes hypersensitivity and toxicity in many patients. Additional studies have shown that Cremophor® EL also causes leaching from polyvinylchloride (PVC) tubing, delivering diethylhexylphthalate (a potential carcinogen) to the patient during intravenous administration. Because of these concerns with currently marketed intravenous cyclosporine, it is suggested that oral cyclosporine be considered as an alternative treatment (Venkataramanan et al., 1986; Volcheck & Van

Dellen, 1998). Lactide and ε-caprolactone biodegradable microspheres loaded with cyclosporine were shown by Li et al. (2005) to potentially deliver steady levels of cyclosporine for several weeks while bypassing metabolic variations seen in convention oral dosing. After intramuscular injection in mice, similar results were seen as tacrolimus biodegradable microspheres maintained steady immunosuppressive blood levels for 2 weeks (Wang et al., 2004). By eliminating toxic solubility enhancers and slowing drug release rate, these types of extended release formulations become increasingly interesting for systemic immunosuppressive therapy.

DELIVERY SYSTEMS INTENDED FOR IMPLANTATION

Using similar prolonged release strategies, implantable polymeric devices have been designed for extended immunosuppressive release in corneal transplant patients. Reservoir corneal implants of cyclosporine have been tested in equine and rabbit models, showing potential to provide sustained release for 3–5 years (Wnek & Bowlin, 2004). Specifically, when glycolide-co-clatide-co-caprolactone was used as a polymeric carrier for sustained release of tacrolimus in a rabbit corneal transplant model, therapeutic drug levels were maintained for over 180 days (Shi, Liu, Xie, & Wang, 2005). Similar results were seen for shorter release times with rapamycin-loaded chitosan/polylactic acid nanoparticles in corneal-transplanted rabbits (Yuan et al., 2008). This methodology is interesting for solid organ transplant recipients as well, potentially allowing for long-term localized therapy. Concern over invasive implantation procedures becomes a mute point in immunosuppression for transplant patients, as these implants could be inserted during transplantation surgery. Further concerns over implant safety and clearance/metabolic variability convey the need for additional studies in this method of immunosuppressive delivery.

DELIVERY SYSTEMS INTENDED FOR THE PULMONARY ROUTE OF ADMINISTRATION

In an effort to localize immunosuppressive effects of calcineurin inhibitors, many groups have developed and investigated these drugs for inhalation. This strategy for targeting the deep lung tissue should provide high local drug concentrations while limiting drug concentrations systemically, in turn reducing the toxic effects imparted by these medications. Cyclosporine, having been used for prevention of transplant allograft rejection for 25 years, was an obvious candidate for development into a formulation for aerosolization.

Cyclosporine

Early research in pulmonary-targeted immunosuppression via cyclosporine aerosol delivery studied the drug's efficacy and tolerability by this delivery method. A basic formulation where pure drug was dissolved in ethanol was produced so that drug aerosolization could occur. Although ethanol is used in many pressurized metered dose inhaler (pMDI) and nebulization formulations and is generally recognized as safe (GRAS) for inhalation at low levels, at high levels it has been shown to be irritating to the lungs and may cause changes in lung function (Zuskin, Bouhuys, & Sari, 1981). Regardless, cyclosporine in ethanol has been dosed in both animals and humans and has shown promising results in comparison to traditional immunosuppressive therapy. In patients experiencing acute cellular rejection, aerosolized cyclosporine (300 mg) was administered as a rescue therapy daily for 10 days, followed by maintenance dosing at three doses per week. Although standard rescue therapy of pulse methylprednisolone or equine lymphocyte immune globulin proved unsuccessful in all 18 patients, aerosolized cyclosporine was effective in 14 of the 18 (Keenan et al., 1997). Additionally, patients displayed improvements in lung function and no significant change in renal function. In lung-transplanted and nontransplanted experimental animal models, aerosolized cyclosporine showed superiority over intramuscular and subcutaneous injections (Mitruka et al., 1998, 2000). At the same dosing level, the area under the concentration versus time curve (AUC) of lungs in aerosol-dosed rats was three times that of rats receiving intramuscular injection. In an allogeneic rat lung transplant model, an 80% greater dose was required in injected rodents as compared to aerosolized rodents to prevent graft rejection. Furthermore, generally lower blood levels were seen in the aerosolized group at equivalent doses.

More recently, cyclosporine has been reformulated in propylene glycol, which is less irritating to the lung mucosa. In a 28-day safety study in rats and dogs, animals received as much as 2.7 times the equivalent maximum human dose (Wang et al., 2007). At these high dosing levels, no unexpected systemic toxicity or respiratory toxicity was seen in either aerosolized cyclosporine in propylene glycol or propylene glycol alone. Additionally, cyclosporine lung concentration in rats was found to be 18 times greater than in the blood at given time intervals. Added viscosity of this solubilizing agent presents another caveat to dosing to the lungs, in that a specific air-jet nebulizer and air compressor must be used. Increased viscosity in pulmonary formulations can lead to high variability in mass median aerodynamic diameter (MMAD), total output, and respirable fraction (RF%) between different nebulizer systems. Characterization of aerosolized cyclosporine in propylene glycol has been performed with the Aerotech II in combination with a high-flow compressor, showing a MMAD of 1.6 μm where 90% of the aerosol produced is below 5.0 μm (Corcoran, 2006). This method of aerosolization has been used in clinical trials of this formulation, ensuring consistent aerosol characteristics in the dose delivered to the patient. While clinical study samples size are small due to the infrequency of pulmonary transplant, several studies have been preformed for evaluation of this formulation in lung transplant recipients. In evaluation of the deposition and absorption rate of aerosolized cyclosporine, Burckart et al. (2003) concluded that the aerosolized drug displayed biphasic absorption shown by the rapid initial absorption into the blood ($t_{1/2}$ = 0.73 h) until 6 h after dosing

when slower absorption after was noted ($t_{1/2}$ = 16.2 h). It was hypothesized that the prolonged retention of cyclosporine in the lung might be due to macrophage uptake or lipophilic interactions between cyclosporine and phospholipids found in pulmonary surfactant and the alveolar membrane (McAllister, Alpar, Teitelbaum, & Bennett, 1996). Using radiolabeling and forced expiratory volume in 1 s (FEV_1), a linear relationship was developed showing a dose-dependent relationship between posttransplant lung function and drug deposited in the lower airways. Improvement in lung function was seen over a 2-year span in patients where dosing resulted in 5 mg of drug deposition in the lung periphery (Corcoran et al., 2004). In a study of patient survival after lung transplantation, conventional immunosuppressive therapy was given to 51 control patients, while 39 cases received cyclosporine aerosol in addition to conventional therapy. Estimates provided by Kaplan–Meier survival analysis showed a 4.5-year mean survival in patients receiving additional aerosol dosing, whereas the control group mean survival was only 2.3 years (Iacono et al., 2004). In a large efficacy study involving lung transplant patients, a randomized single-center trial was performed involving 30 aerosol placebo-dosed patients and 28 aerosol cyclosporine patients, all of which received oral tacrolimus, azathioprine, and prednisone for immunosuppressive management in addition to aerosol therapy. Results of this trial showed that while having no significant effect on the incidence of acute allograft rejection, both patient survival (47% placebo vs. 11% cyclosporine) and chronic rejection rates were significantly improved in the patients receiving aerosolized cyclosporine (Iacono et al., 2006) (Figure 4). Interestingly and contrary to previous studies, there seems to be no relationship in this study between acute rejection and chronic rejection in the aerosol-dosed group. The authors theorize that as aerosolized cyclosporine contributes minimally to blood concentrations, there is less immunosuppressive effect on vascular lung tissue. Acute rejection, which is histologically characterized by lymphocytic infiltrates in and around vessels, might see little therapeutic effect with the addition of aerosol treatment.

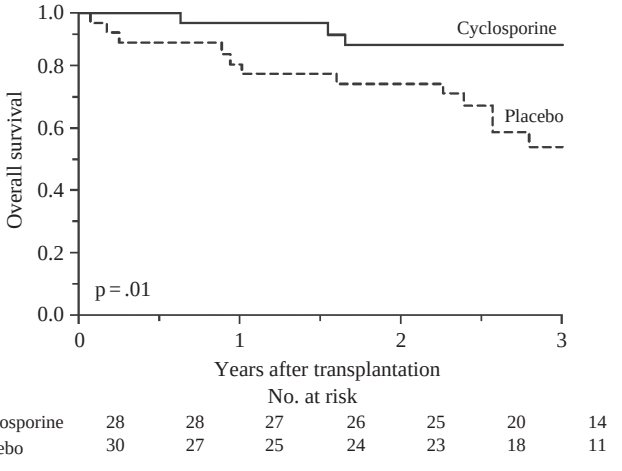

FIGURE 4. Survival of lung transplant recipients receiving aerosolized cyclosporine in addition to normal immunosuppressive maintenance therapy. (Reprinted with permission from Iacono et al., 2006.)

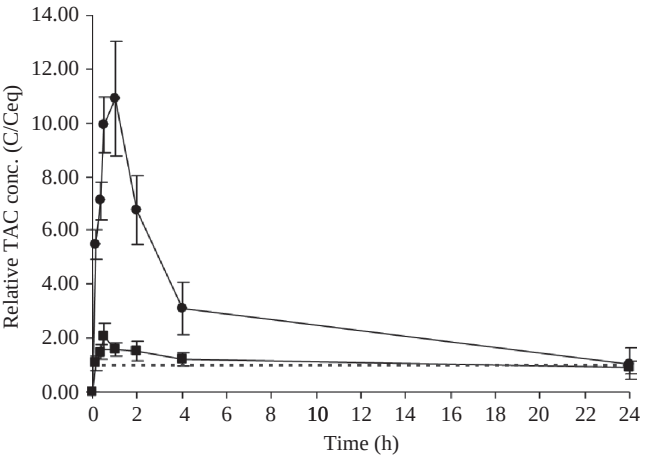

FIGURE 5. Supersaturated dissolution profile for (●) amorphous ultra-rapid freezing (URF) composition tacrolimus (TAC) : lactose (1:1); (■) crystalline URF composition TAC alone; and (---) equilibrium solubility of TAC in the dissolution media fluids (SLF) containing 0.02% DPPC at 100 rpm and 37°C. (Reprinted with permission from Sinswat et al., 2008.)

The underlying issue in the FDA approval of what seems to be much improved targeted delivery method is the lack of homogeneity and small size of patient populations in trials. With only 22% of lung transplant centers performing more than 20 transplants a year (Trulock et al., 2007), the need for a unified multicenter effort becomes apparent. This, however, only compounds the problems of different immunosuppressive treatment protocols (i.e., induction, maintenance, adjuvant, and steroid therapies) and imbalances between treatment and placebo groups (DeCamp, 2006). These issues, combined with a relatively small market outlook, have led Novartis AG, the developer of this orphan drug, to sell rights to APT Pharmaceuticals (Burlingame, CA, USA) which is expected to begin phase III trials this year.

In an effort to enhance the solubility of cyclosporine without the use of a lung irritating solvent or highly viscous agent, liposomal cyclosporine for inhalation was developed by Gilbert and coworkers (Gilbert, Black, Bennick, Montgomery, & Knight, 1997). Preparation of cyclosporine in dilauroylphosphatidylcholine (DLPC) consisted of dissolving both excipients in *t*-butanol, followed by solvent removal and redispersion in water. In addition to solubility enhancement, liposomal delivery may offer the added benefit of drug retention in the lungs. Liposomal aerosol deposition in a dog model showed lung retention of approximately 120 min after a 25-mg dose (Letsou et al., 1999).

Tacrolimus

As documented in laboratory and clinical studies, both oral and intravenous tacrolimus formulations have shown increased potency and efficacy over cyclosporine. Subsequently, many

immunosuppressive protocols now have replaced the calcineurin inhibitor cyclosporine with tacrolimus. In keeping with this trend, researchers are now investigating the efficacy and safety of aerosolized tacrolimus in transplanted and nontransplanted animal models. The goal, as with inhaled cyclosporine, is to enhance immunosuppressive effects in the lung while limiting toxicity systemically. In anticipation of the interest in using tacrolimus for targeted pulmonary delivery in transplantation as well as asthma therapy, Fujisawa (now Astellas Pharma Inc., Tokyo, Japan) developed and patented a pMDI formulation containing tacrolimus, miglyol 812 (a lipophilic solubilizer), and HFA-227 (propellant) (Murata, Shiomojo, Tokunaga, & Hata, 2002). In the first study incorporating inhaled tacrolimus, Ingu et al. (2005) investigated the efficacy of aerosolized tacrolimus in lung allograft immunosuppression and compared with immunosuppression afforded by tacrolimus intramuscular injection. In both groups, histopathological evaluation and suppression of cytokine expression denoted local immunosuppression in the transplanted lungs. As is the case with inhaled cyclosporine, inhaled tacrolimus was found to provide the same efficacy in the lung as intramuscular injection while showing reduction in systemic blood levels (<0.5 ng/mL inhaled and 5.2 ng/mL intramuscular). Subsequent investigations have quantified tacrolimus deposition levels in the lungs by a microparticle enzyme immunoassay method in transplanted rats with dose administered through intramuscular injection and inhalation. Significant immunosuppression was observed histologically in animals having a tacrolimus lung concentration of 270.4 ng/mL 1 h after sacrifice (Ide et al., 2007). In addition to potential for lung transplant therapy, inhaled tacrolimus delivered by pMDI has also been investigated for inflammation reduction in egg-albumin-challenged guinea pigs (Morishita et al., 2005). Another formulation for aerosolization of tacrolimus has been studied by Schrepfer et al. Also in solubilized form, tacrolimus is aerosolized with 70% ethanol solution and studied for its inhibition of obliterative airway disease in tracheal-transplanted rats (Schrepfer, Deuse, Fink, Haddad, & Robbins, 2008; Schrepfer, Deuse, Hoffman et al., 2007; Schrepfer, Deuse, Reichenspurner et al., 2007). Much like previous formulations, findings demonstrated that aerosolized tacrolimus can achieve potentially immunosuppressive concentrations in the lungs while avoiding excessive systemic levels. Upon histopathological examination, tracheal grafts did show immunosuppression in both oral and aerosol groups. Interestingly, upon cessation of aerosol and oral treatments, tracheal influx of inflammatory cells is much more rapid in the aerosol-treated group due to lower systemic immunosuppression. Another novel formulation involving dispersed tacrolimus nanoparticles has been reported for prevention of lung allograft rejection via pulmonary delivery. This formulation offers the benefit of supersaturating pulmonary fluid without the use of lung irritating solvents. In vitro efficacy was shown by lymphocyte suppression in mixed leukocyte culture (MLC) and mitogen stimulation assays (MSA) (Peters, Purvis, Pollack, Angel, & Williams, 2007). A dispersion of tacrolimus and lactose (1:1) nanoparticles has been evaluated in in vitro-dosed to mice evaluated for blood and lung levels (Sinswat, Overhoff, McConville, Johnston, & Williams III). Preliminary studies involving pulmonary dosing of this dispersion in a rat lung transplant model showed that high lung levels are achieved while whole blood concentration remains low. Additionally, it was noted that drug concentration in transplanted allografts showed prolonged therapeutic levels in comparison to nontransplanted lungs.

Although no successful lung-transplanted animal studies are shown in the literature, liposomal tacrolimus has been synthesized using dipalmitoylphosphatidylcholine (DPPC) for potentially targeting lung immunosuppression (Canadas et al., 2004). As in pulmonary liposomal formulations of cyclosporine, tacrolimus liposomal formulations may allow for longer drug residence times after single dosing.

Corticosteroids

Oral or intravenous administration of corticosteroids in conjunction with a calcineurin inhibitor and antimetabolite has long been standard practice for maintenance immunosuppressive therapy. Inhaled corticosteroids used in the treatment of asthma and chronic obstructive pulmonary disease induce an anti-inflammatory response, suggesting possible application for immunosuppression in lung transplant patients. In spite of these observations, conflicting results have been seen in inhalation of corticosteroids for prevention of lung allograft rejection. Early success was seen in treating bronchiolitis obliterans syndrome (BOS) with inhaled budesonide and fluticasone (Speich, Boehler, & Russi, 1997; Takao, Higenbottam, & Audley, 1995). More recently, in a clinical trial investigating reduction of lymphocytic bronchiolitis in transplanted lungs, inhaled budesonide was shown to improve lung function (De Soyza, Fisher, Small, & Corris, 2001); however, the absence of histological evaluation and long-term patient success rates leave cause for further analysis. A more recent placebo-controlled clinical trial found that inhaled fluticasone propionate dosing twice daily for 3 months did not significantly prevent BOS in 30 lung transplant patients tested (Whitford et al., 2002). Potential reasons for the inefficacy of this targeted therapy might have been the early onset of BOS (approximately 6 months after transplant), known difficulties with pMDI use, or inadequate drug deposition in small airways. In a specific case cited by Naef and coworkers, comedication of inhaled fluticasone propionate and prophylaxis itraconazole resulted in Cushing's syndrome in some lung-transplanted patients. It was deduced that inhibition of cytochrome P450 by itraconazole reduced clearance of fluticasone, causing accumulation in lung tissue and increased blood concentrations (Naef et al., 2007). Based on these few studies, it becomes apparent that further work investigating early intervention with various corticosteroid formulations for prevention of BOS is necessary.

TABLE 1
Calcineurin Inhibitor Delivery Methods for Transplant Immunosuppression

Route	Calcineurin Inhibitor	Delivery Technology	Improvements	Brand Name	References
Oral	Cyclosporine	Drug solubilized in emulsion	N/A	Sandimmune®	(Metha et al., 1988)
		In situ microemulsion	Improved bioavailability, less variability	Neoral®	(Holt & Johnston, 1997)
		Charged nanoparticles	Improved bioavailability	—	(El-Shabouri, 2002)
		Polycaprolactone nanoparticles	Target lymphocytes, improved bioavailability	—	(Varela, 2001)
		Polymeric micelles	Caco-2 permeability, enhanced solubility	—	(Francis et al., 2005)
		pH-sensitive nanoparticles	Targeted area of GI tract, improved bioavailability	—	(Dai et al., 2004)
		Polymeric microspheres	Controlled release, enhanced solubility	—	(Gref et al., 2001)
		Stabilized amorphous particles	Enhanced solubility	—	(Chen et al., 2002; Liu et al., 2006)
	Tacrolimus	Stabilized amorphous particles	N/A	Prograf®	(Yamashita et al., 2003)
		Hydrophilic cyclodextrin	Enhanced solubility	—	(Arima et al., 2001)
		Biodegradable nanoparticles	Targeted area of GI tract	—	(Lamprecht et al., 2005)
		pH-sensitive nanoparticles	Targeted area of GI tract	—	(Meissner et al., 2006)
Parenteral	Cyclosporine	Drug solubilized in Cremphor® EL	N/A	Sandimmune®	—
		Liposomal	Tissue targeting, longer half life, reduced toxicity	—	(Arulsudar et al., 2003)
		Polymeric micelles	Reduced toxicity, prolonged release, enhanced solubility	—	(Aliabadi et al., 2005; Guo et al., 2005)
		Biodegradable microspheres	Sustained release, reduced toxicity	—	(Li et al., 2005)
	Tacrolimus	Drug solubilized in emulsion	N/A	Prograf®	—
		Biodegradable microspheres	Prolonged release	—	(Wang et al., 2004)
		Liposomal	Tissue targeting, longer half life	—	(Moffatt et al., 1999)
Implant	Cyclosporine	Polymeric reservoir	Sustained release	—	(Shi et al., 2005)
Pulmonary	Cyclosporine	Dissolved in ethanol	High lung levels, low systemic levels	—	(Keenan et al., 1997; Mitruka et al., 1998, 2000)
		Dissolved in propylene glycol	Less irritating to lung	Pulminiq™	(Iacono et al., 2004, 2006; Wang et al., 2007)
		Liposomal	Enhanced solubility, lung retention	—	(Gilbert et al., 1997)
	Tacrolimus	Metered dose inhaler	High lung levels, low systemic levels	—	(Ingu et al., 2005; Ide et al., 2007)
		Ethanol solution	Suitable for nebulization	—	(Schrepfer et al., 2007)
		Dispersed amorphous nanoparticles	Less irritating, enhanced solubility	—	(Sinswat et al., 2008)
Sublingual	Tacrolimus	Prograf® capsule content	Reduced metabolism, high systemic levels	—	(Reams et al., 2001; Romero et al., 2008)

Antifungal Prophylaxis

In addition to immunosuppression, lung transplant patients often require prophylactic antifungal therapy to assist a weakened immune system in defending against invasive fungal infections. Many of these treatments are delivered orally or intravenously for transplant recipients; however, novel inhaled antifungal formulations have been used to target infection in lung allografts. Abelcet®, a lipid complex of amphotericin-B, has been dosed to single and double lung transplant recipients to determine drug deposition. As expected, lower deposition was seen in the native lung of single transplant patients because of lower ventilation. Clinical data has shown that *Aspergillus* infections may originate from the native lung in patients with single lung transplants (Westney et al., 1996); consequently, therapeutic drug levels in this region are important. It is suggested that techniques for targeting lower ventilated areas should be evaluated to overcome this issue (Corcoran et al., 2006; Mohammad & Klein, 2006). In another study, efficacy of pentamide prophylaxis was demonstrated in lung transplant patients for the prevention of *Pneumocystis jeroveci* (formerly *carinii*) pneumonia (Nathan, Ross, Zakowski, Kass, & Koerner, 1994). This treatment was used instead of sulfamethoxazole-trimethoprim to prevent sulfa-related allergy. Future studies exploiting pulmonary delivery of antifungals in lung transplant cases are needed before this mode of therapy can be commonly used in clinical settings.

DELIVERY SYSTEMS INTENDED FOR THE SUBLINGUAL/BUCCAL ROUTE OF ADMINISTRATION

Cystic fibrosis, being one of the main indications for pediatric lung transplantation, is a disease that affects many organs, including the lungs and the gastrointestinal tract. It is characterized by production of thick mucus in the lungs, recurrent pulmonary infections, and diminished pancreatic enzyme production. Consequently, orally administered lipophilic substances tend to be poorly absorbed through the intestinal mucosa. In the case of orally administered immunosuppressants after transplantation (i.e., tacrolimus and cyclosporine), a further reduced bioavailability might be expected (Knoop et al., 2005). An alternative has been proposed to obtain therapeutic blood levels in transplanted patients by sublingual drug delivery (Reams, Rea, Davis, & Palmer, 2001). Because of the variations in drug absorption after sublingual administration, only 67% of the blood samples taken in six lung-transplanted patients with cystic fibrosis showed therapeutic levels. In a recent case report involving a kidney transplant patient, a contraindication arose that eliminated oral tacrolimus as a treatment option. For approximately 1 month, therapeutic levels (between 7 and 15 ng/mL) of tacrolimus were targeted relatively successfully by taking the contents of oral tacrolimus and administering sublingually (Romero et al., 2008). The contents of oral tacrolimus (Prograf®), HPMC:tacrolimus matrix, could be expected to swell in the oral cavity and release amorphous tacrolimus into the saliva. Additionally, in pediatric liver transplants, buccal administration of tacrolimus suspension was used to promote patient compliance and prevent interference with gastrointestinal function. When compared to trough levels achieved by nasogastric tube (NGT) administration, buccal administration showed comparable therapeutic levels in the target range (31% buccal, 24% NGT) (Goorhuis, Scheenstra, Peeters, & Albers, 2006). More investigation into this method of calcineurin inhibitor delivery is needed, including design of a mucoadhesive controlled release formulation and comprehensive pharmacokinetic evaluation in animal models.

CONCLUSION

As new immunosuppressive chemicals are developed, delivery methods to maximize and target their therapeutic effect must be developed. In the case of lung transplantation, unique challenges confront clinicians as they try to avoid allograft rejection through suppression of the immune system while not completely disabling the body's ability to protect itself from invasive infection. Likewise, unique opportunities for targeted drug delivery by pulmonary delivery may allow for local therapeutic effects, while limiting systemic toxicity. As further discoveries are made in new immunosuppressive chemical entities and subsequent investigations are conducted in safe and efficacious ways to delivery them, lung transplant patient quality of life and survival rates will continue to improve.

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