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Title: Optimizing immunosuppression with mTOR inhibitors in renal transplant recipients

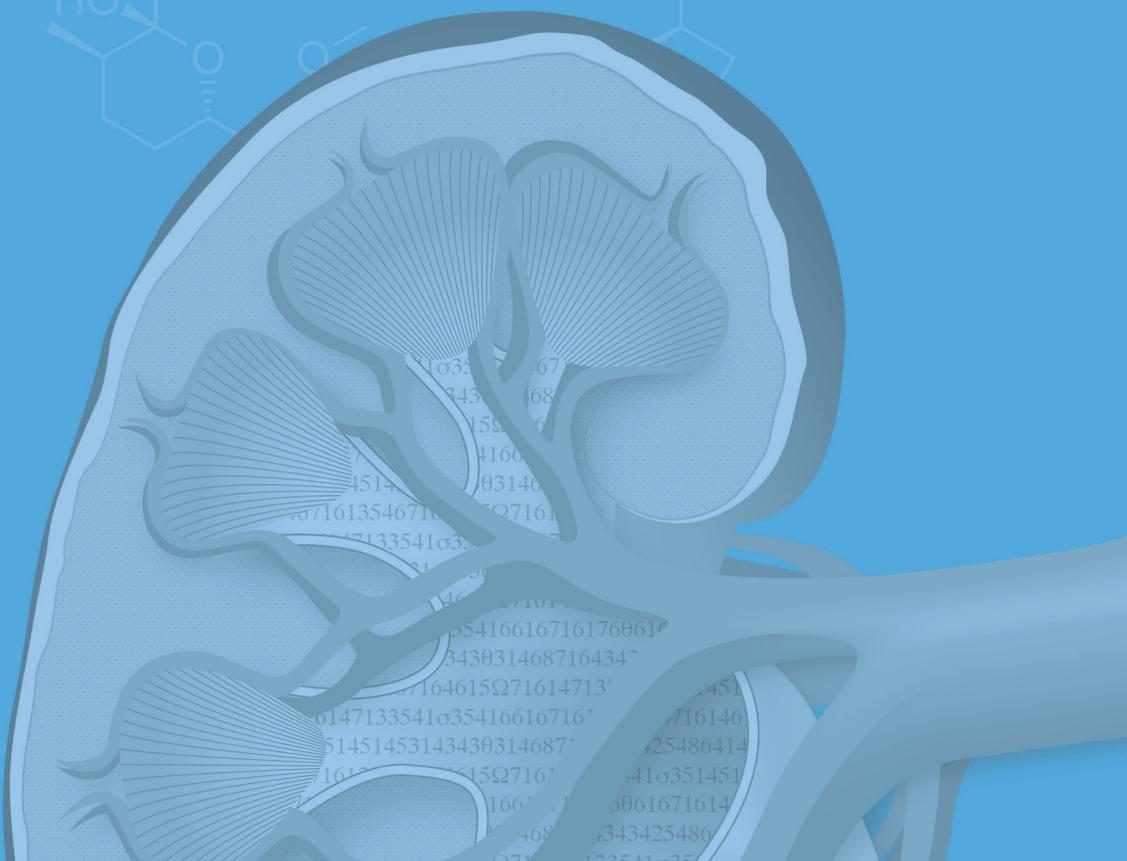
Issue Date: 2014-11-18

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Clinical pharmacokinetics and pharmacodynamics of mTOR inhibitors in renal transplantation

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Submitted



Abstract

The mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus are a relatively new therapeutic group in renal transplantation and have shown their efficacy in recent trials. Their main advantage compared to the calcineurin inhibitors cyclosporine and tacrolimus are their relative lack of nephrotoxicity. Sirolimus differs from everolimus mainly in pharmacokinetic characteristics such as elimination half-life and bioavailability. The oral mTOR inhibitors exert both highly variable inter- and intra-individual pharmacokinetics. They are metabolized by CYP3A4, CYP3A5 and CYP2C8 enzymes and are substrates for P-glycoprotein and share similar pharmacodynamics. Polymorphisms in genes coding for these enzymes might be of interest for optimizing immunosuppressive therapy. The most important side effects of sirolimus and everolimus are thrombocytopenia, leukopenia, hypercholesterolemia, diarrhea and although rare but potentially life threatening interstitial pneumonia. The narrow therapeutic window of mTOR inhibitors, together with high variability in pharmacokinetics, makes therapeutic drug monitoring essential for individualizing the dose and thereby preventing toxicity or rejection. The main future challenge is to further optimize mTOR inhibitor based immunosuppressive therapy.

Introduction

In the last 30 years considerable progress has been made in the field of renal transplantation with regard to immunosuppression, since the calcineurin inhibitors (CNIs) cyclosporine and later on tacrolimus came available to the clinic. However, despite this success, calcineurin inhibitors are also associated with severe toxicity such as acute and chronic nephrotoxicity [1,2]. In an effort to find new immunosuppressive drugs without or less nephrotoxicity mTOR inhibitors were introduced in renal transplantation. The mTOR inhibitors sirolimus (Rapamune®) and everolimus (Certican®) are potent orally administered immunosuppressive agents. Both are derived from a macrocyclic lactone produced by *streptomyces hygroscopicus* recovered from Easter Island [3,4]. Similarities exist between other macrocyclic lactones such as erythromycin and tacrolimus with regard to their chemical structures. Although highly active against *Candida Albicans* sirolimus was commercially launched for its immunosuppressive potency discovered in animals [5,6] and later suggested for clinical renal transplantation [7]. Everolimus is a derivative of rapamycin (sirolimus) and was developed for prevention of acute and chronic rejection of solid organ transplants. Instead of a hydrogen atom at position 40 it has a 2-hydroxyethyl chain (Figure 1a en 1b) substitution which improves the solubility and bioavailability of the drug [4].

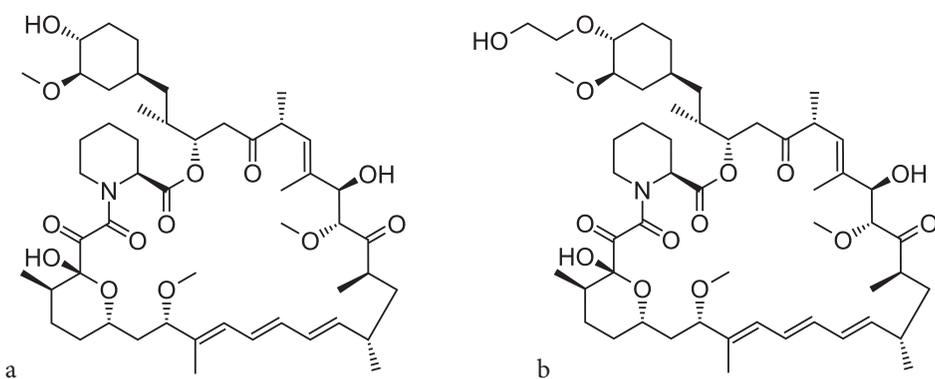


Figure 1: Chemical structure of Sirolimus and Everolimus.

In the past years mTOR inhibitors were only prescribed in combination with cyclosporine and steroids since a synergistic effect and different mechanism of action is present compared to CNIs [8,9], but as a result of the damaging effects of cyclosporine on the donor kidney everolimus is now tested in absence of cyclosporine in clinical trials [10,11].

Meanwhile a combined CNI everolimus regimen has proven its effectiveness in a number of clinical trials [12,13]. This systematic review gives an oversight on current knowledge of clinical pharmacokinetics, pharmacodynamics and pharmacogenetics of mTOR inhibitors in renal transplantation.

Literature search methods and results

An initial Pubmed search was conducted to find all available literature concerning clinical pharmacokinetics and pharmacodynamics of mTOR inhibitors using the following search criteria: {(Everolimus OR SDZ-RAD OR 40-O-(2-hydroxyethyl)-rapamycin OR “SDZ RAD” OR Certican OR “RAD 001” OR RAD001 OR Sirolimus) AND (pharmacokinetics OR pharmacokinetic* OR “Area Under Curve” OR “Biological Availability” OR “Metabolic Clearance Rate” OR “Therapeutic Equivalency” OR “Tissue Distribution” OR “Pharmacogenetics” OR “Pharmacogenetic”* OR “Pharmacodynamics” OR “Pharmacodynamic*”) AND (renal transplantation OR kidney transplant) NOT oncology NOT tumors}. This resulted in 300 articles derived from Pubmed, subsequently the same search criteria was used for Web of Science (316 articles), EMBASE (102 articles) and Cochrane (2 articles). Articles were limited to those written in the English language. After removing duplicates 525 remained were reviewed for relevancy. 344 articles remained after evaluating the titles and abstracts. Focusing on pharmacokinetics, pharmacodynamics, therapeutic drug monitoring and side effects led to a total of 109 obtained full text articles which were used to summarize these findings.

Pharmacokinetics

Absorption

Sirolimus

Sirolimus is rapidly absorbed after oral administration with an average maximum blood concentration (c_{\max}) (SD) of 40.5 ± 22.2 $\mu\text{g/L}$ when administering a dose of 2.5 mg. The maximum concentration is reached after 2.7 ± 2.1 hours (t_{\max}) and is dependent on the dose administered (0.5 - 6.5 mg) [14]. In patients receiving an immunosuppressive regimen of cyclosporine and prednisone with single or multiple doses of sirolimus, sirolimus

was absorbed rapidly with average t_{\max} (%CV): 1.6 (81%) and 1.4 (85%) hours after administration respectively [14,15]. Steady state was reached within 14 days. Its steady state maximum concentration and area under the blood concentration versus time after administration curve (AUC) were dose proportional over the dose range of 0.5 – 6.5 mg/m² once daily [14]. The absolute bioavailability of sirolimus in humans is unknown, however it has been estimated to be around 14% and highly variable (range 10.9 – 16.9%) [16]. Results from preclinical studies also showed a low bioavailability (10%) [17]. Food intake strongly affects the bioavailability of sirolimus; a 35% increase in AUC after a fatty meal was observed in a clinical trial, but absorption was more slowly [18]. Therefore sirolimus should be administered consistently in individual patients, either with or without meals to assure consistent exposure. In a cohort of 150 renal transplant patients, no correlation was found between sirolimus concentrations and bodyweight, gender, age or dose [19]. Currently two formulations are available in the clinic: a tablet and a non-aqueous oral solution. In a comparative study, values of c_{\max} for the solution were significantly greater compared to the tablet. Moreover c_{\max} for the tablet observed on day 1 was significantly greater compared with days 30 and 90. Furthermore t_{\max} was significantly greater for the tablet. However average sirolimus pharmacokinetic parameters were not significantly different when comparing both formulations, only t_{\max} was slower for tablet administration but no clinically relevant differences were found [20]. Similar results were found in a conversion study from one formulation to the other [21]. Intestinal CYP3A metabolism and intestinal P-glycoprotein (P-gp) counter transport, intestinal membrane permeability and hepatic first-pass affect bioavailability most likely also influence sirolimus absorption since sirolimus is a substrate for these enzymes and transporters [22] as schematically shown in Figure 2. Pharmacokinetic parameters are clearly influenced by the presence and timing of co-administration of cyclosporine [23] since both drugs are substrate and inhibitors of the same metabolizing enzymes [22,24].

Everolimus

Everolimus is rapidly absorbed after oral administration with an average c_{\max} (SD) of 45 (\pm 21) μ g/L when administering a dose of 2.5 mg. The maximum concentration is reached after 1.3 ± 0.4 hours after dose administration and is dependent on the dose administered (0.25 - 25mg) [12]. In a study with patients with immunosuppressive regimen of cyclosporine and prednisone receiving multiple doses of everolimus, everolimus was absorbed rapidly (average t_{\max} 2 hours), Steady state was reached within 7 days. Steady

state maximum concentration and AUC were dose proportional over the dose range of 0.5 – 2 mg twice daily [25]. The bioavailability of everolimus in animal models is low with an amount of around 16% [26,27] but slightly higher than sirolimus. Absolute bioavailability data of everolimus is not available since no intravenous formulation exists but intra- and inter-individual variability is high [25]. Currently two everolimus formulations are on the market; a solid tablet and a dispersible tablet, the latter initially developed for pediatrics. The bioavailability of everolimus from the dispersible tablet was found to be 10% lower relative to the conventional tablet [28]. As sirolimus, the relative bioavailability of everolimus is affected by food since food affects the absorption [29,30]. In healthy subjects receiving a single 2 mg dose it was found that when combining with a high-fat meal t_{max} was delayed by a median 1.25 hours. Furthermore c_{max} was reduced by 60% and reduced AUC by 16%. In renal transplant recipients, a high-fat meal delayed t_{max} by a median 1.75 hours and reduced c_{max} by 53% and AUC by 21%. Everolimus trough levels showed no food effect, while peak-trough fluctuation was lowered by 52%. [30]. Everolimus should therefore be consistently administered with or without food in individual patients. Intestinal CYP3A metabolism and intestinal P-glycoprotein activity, Intestinal membrane permeability and hepatic first-pass affect bioavailability probably play a large role in the absorption of everolimus since everolimus is also substrate for CYP3A4, CYP3A5, CYP2C8 and P-gp [31] as schematically shown in Figure 2. Co-administration of cyclosporine leads to an altered metabolism since both drugs are substrate and inhibitors of the same metabolizing enzymes [24,32].

Distribution

Sirolimus

Sirolimus is a hydrophobic compound, is extensively distributed to various organs with an steady state distribution volume (V_{ss}) of 7-19 L/kg [15,33] and is more partitioned into red blood cells (up to 95%) than plasma (3%) and lymphocytes 1% [16,34]. Whole blood is therefore the matrix of choice for therapeutic drug monitoring. Plasma to blood ratio was found to be 35:1 in a group of 36 stable renal transplant recipients and considerable inter-individual variability (CV of 52%) was reported [15]. Sirolimus was primarily associated with non-lipoprotein fractions in plasma [34]. In studies in rats considerable accumulation of sirolimus in the heart, kidney, intestine, and testes were found [35]. Whether this is the same in humans has not been investigated.

Everolimus

The less hydrophobic compound everolimus is at therapeutic concentrations for more than 75% partitioned into red blood cells and 75% of the plasma fraction is bound to plasma proteins [25]. The estimated volume of distribution for a 71 kg patient is at steady state 110 L and is increased with 1.14 for each kilogram increase in body weight [29]. In rats the highest binding potential was observed in thymus, lungs and spleen [27]. In monkey lung transplant recipients the highest concentrations were found in gall bladder, transplant lung, cerebellum, kidneys and spleen [36]. Data in humans is not available.

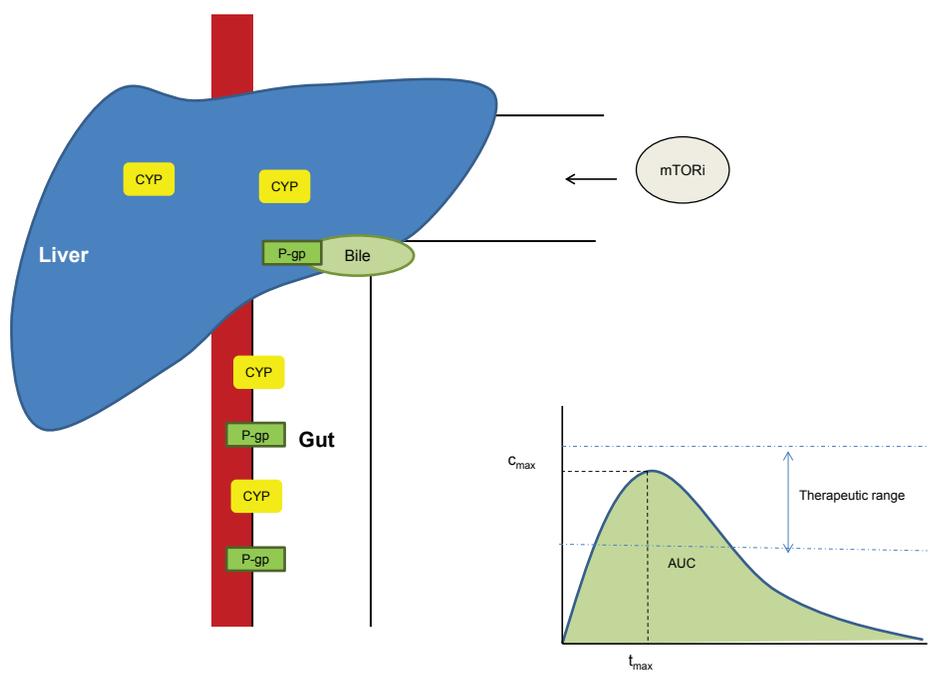


Figure 2: Schematic representation of oral administration of mTOR inhibitors, interaction with metabolic enzymes and effect on blood levels. AUC, area under the blood concentration vs time after dose administration curve; CYP, cytochrome P450 enzymes (CYP3A4, CYP3A5 and CYP2C8); C_{max}, maximum blood concentration; mTORi, mTOR inhibitor; t_{max}, time to reach maximum blood concentration.

Clearance

Sirolimus

Sirolimus is primarily metabolized by CYP3A4, but also by CYP3A5 and CYP2C8 [22,37,38]. The large inter-individual variability in metabolism of sirolimus is probably

a reflection of the wide inter-individual variability in expression of these enzymes [39]. Moreover sirolimus is also a substrate for P-glycoprotein [40]. In a population pharmacokinetic analysis of 36 renal transplant patients a wide variability in clearance was found, terminal half-life was 63 hours (27.5%) and apparent oral blood clearance of 8.9 L/hr (38.2%). Elimination was not influenced by dose [15]. In another pharmacokinetic study with 40 stable renal transplant patients clearance was found to be 0,208 (45%) mL/hr/kg; terminal half-life was, 62 (± 16) hours allowing a once daily regimen. Furthermore a loading dose of three times the maintenance dose was suggested to achieve therapeutic concentrations more rapidly [14]. The four main metabolites of sirolimus are 16-O-demethyl-sirolimus, 39-O-demethyl-sirolimus, 27-39-O-di-demethyl-sirolimus and di-hydroxy-sirolimus [41]. The activity of these metabolites seems to be less than 10% of the parent compound [42]. Preliminary results showed that black renal transplant patients had a higher metabolism compared to non-blacks [15]. Furthermore, another study showed significant lower trough concentrations and higher acute rejection rates for black patients [43]. In a study with 18 adult subjects with mild to moderate hepatic impairment and 18 healthy control subjects, mean whole-blood sirolimus weight-normalized and oral-dose clearances (CL/F) were significantly decreased in subjects with mild to moderate hepatic impairment by 31.8% and 36.0%, respectively, compared with controls after administration of a single 15 mg oral solution dose [44].

Everolimus

Everolimus is also metabolized by CYP3A4, CYP3A5 and CYP2C8 and is a substrate for P-gp [22,31,45]. In a “first into human” study with single everolimus doses the elimination half-life and ranged from 24 to 35 h across the doses in the range of 0.25 – 25 mg. The average AUC ($\mu\text{g}\cdot\text{h}/\text{L}$) ranged from $171 \pm 50 \mu\text{g}\cdot\text{h}/\text{L}$ for the 0.75 mg group to $2400 \pm 608 \mu\text{g}\cdot\text{h}/\text{L}$ for the 25 mg group [46]. In a population pharmacokinetic analysis of 673 patients [29] the following pharmacokinetic parameters were found: the apparent average clearance for a 44 years Caucasian patient old weighing 71 kg was 8.8 L/h ($\pm 27\%$) with a central distribution volume of 110 L ($\pm 36\%$). Everolimus pharmacokinetics is greatly affected by cyclosporine which inhibits CYP3A4 [29]. In 8 healthy volunteers, everolimus apparent clearance was 19.4 L/h in absence of cyclosporine [47]. Therefore renal transplant patients probably also have a higher clearance in cyclosporine free regimens. Everolimus pharmacokinetics was not affected by age, sex and weight in adults. Asian ethnicity did not affect everolimus clearance. Patients indicated as black had a 20% higher clearance

compared to non-black patients [29,48]. Since everolimus has a rapid clearance, everolimus requires twice-daily administration in contrast to sirolimus. The four main metabolites of everolimus are: hydroxyl-everolimus, dihydroxy-everolimus, dimethyl-everolimus and a ring opened form of everolimus [37]. In a population pharmacokinetic study the inter-individual variability in clearance was reduced to 27% after accounting for the covariates [29]. The intra-individual variability and residual error was 31%. In a multicenter randomized double blind study of 101 renal transplant patients inter-individual variability in terms of AUC for everolimus was 85.4%, intra-individual, inter-occasion variability was 40.8% [49], implicating the need for therapeutic drug monitoring. In a study investigating the influence of hepatic impairment on everolimus pharmacokinetics it was found that the apparent clearance of everolimus was significantly reduced by 53% in subjects with moderate hepatic impairment compared with healthy subjects. This was reflected by a 115% higher AUC (245 +/- 91 versus 114 +/- 45 $\mu\text{g}\cdot\text{h}/\text{L}$) and 84% prolonged half-life (79 +/- 42 versus 43 +/- 18 hours) [47]. Furthermore a significant positive correlation of the everolimus AUC with bilirubin level ($r = 0.86$) and a significant negative correlation with albumin concentration ($r = 0.72$) was found. Therefore dose reduction and close TDM may be indicated.

Excretion

Sirolimus

Sirolimus is metabolized through the liver, 91% of sirolimus metabolites are excreted in the bile, only 1.2% is excreted through urine [50].

Everolimus

Everolimus is also metabolized through the liver, after metabolizing approximately 98% is excreted as metabolites in the bile [46].

Drug interactions

Sirolimus

Since sirolimus is metabolized by CYP3A4, CYP3A5 and CYP2C8 and a substrate of P-gp, inhibitors or inducers of these enzyme most likely show pharmacokinetics interactions. In vitro anti-CYP3A antibodies, as well as the specific CYP3A inhibitors troleanomycin and erythromycin, inhibited small intestinal metabolism of sirolimus [22]. In a renal transplant recipient an interaction between dronedarone and sirolimus was reported. A 3 fold

increase of sirolimus trough concentration (38.6 µg/L) was observed 3 days after initiation of dronedarone. If concurrent administration cannot be avoided, close monitoring and a 50-75% dose reduction of sirolimus prior to dronedarone initiation was recommended [51]. Trimethoprim-sulphamethoxazole combination did not affect sirolimus steady state pharmacokinetics in 15 renal transplant recipients [52]. In two case reports rifampicin significantly increased sirolimus pharmacokinetics; the dosage of sirolimus had to be increased, in one case up to six-fold and in the second case up to five-fold, to maintain serum levels after starting the rifampicin [53]. Diltiazem increased sirolimus AUC by 60%, ketoconazole increased sirolimus AUC by 990% and rifampicin reduced sirolimus AUC by 82% in a phase III trial [54]. In a pharmacokinetic analysis of 36 patients cyclosporine did not seem to affect sirolimus pharmacokinetics [15]. In contrast *Cattaneo et al.* reported that concomitant cyclosporine therapy resulted in significantly higher sirolimus trough values compared to concomitant tacrolimus or mycophenolate mofetil therapy [55]. Moreover in another study with 24 stable renal transplant recipients sirolimus AUC and trough levels were consistently and significantly higher when both cyclosporine and sirolimus were administered concomitantly, than when they were administered 4 hours apart indicating a inhibiting effect of cyclosporine on sirolimus pharmacokinetics [23]. Generic and brand name cyclosporine also seem to alter sirolimus pharmacokinetic differently as was reported by Kovarik et al [56]. Finally a twofold increase in cyclosporine AUC was associated with a 63% mean increase in sirolimus AUC in 53 stable kidney transplant recipients [57]. The combination of cyclosporine and sirolimus is synergistic as previously demonstrated in vitro and in vivo in animal transplant experiments [9]. Sirolimus not only increases cyclosporine concentrations in blood but also in the kidney. This interaction may lead to increased cyclosporine associated nephrotoxicity by a mechanism which is still not entirely understood [9]. In a pharmacokinetic study investigating the effect of tacrolimus on sirolimus pharmacokinetics neither pharmacokinetic profiles of sirolimus nor those of tacrolimus were altered by simultaneous administration [58].

Everolimus

Administration of erythromycin, azithromycin, or itraconazole in combination with everolimus (0.75 or 1.5 mg twice daily) resulted in a 22, 18 and 74% lower everolimus clearance compared to everolimus alone [29]. Calcium channel blockers, quinolones and trimethoprim-sulfamethoxazole had no effect on everolimus pharmacokinetics [29]. In 12 healthy subjects, rifampicin co-administration, a CYP3A and P-gp inducer, resulted in a

significantly increased apparent clearance of 172% on average [59]. Co-administration of atorvastatine (CYP3A4 substrate) or pravastatin (P-gp substrate) has no clinically relevant interaction with everolimus as was found in 24 healthy volunteers [60]. Everolimus trough concentrations were significantly elevated in the presence of cyclosporine [61]. In a study with 56 de novo renal transplant recipients received basiliximab, corticosteroid and either immediate or delayed initiation of cyclosporine based on renal function, trough concentrations were significantly lower (3 fold) in absence vs in presence of cyclosporine [61]. In healthy volunteers it was shown that two cyclosporine formulations; neoral[®] and sandimmune[®] had different effects on everolimus pharmacokinetics. Neoral[®] co-administration resulted in significantly greater everolimus AUC compared to sandimmune[®] co-administration 168% vs 74% increase [62]. Co-administration of tacrolimus seems to have a much less pronounced effect than cyclosporine on everolimus pharmacokinetics. No clinically relevant change in everolimus exposure was found [63].

Pharmacodynamics

Mechanism of action

Sirolimus and everolimus share the same mechanism of action (Figure 3). They block Ca²⁺-dependent and Ca²⁺-independent events during G1 phase of the cell cycle, including transduction of second signals delivered by interleukin (IL)-2, IL-3, IL-5 and IL-6. They also block, but to a lesser extent, the signals delivered by fibroblast growth factor, stem cell factor, platelet-derived growth factor, colony-stimulating factor and insulin growth factor. In *in vitro* experiments, sirolimus and everolimus inhibited a variety of mitogen- and antigen driven B- and T-lymphocyte proliferative responses [6,64,65].

Sirolimus and everolimus bind to FK506 (tacrolimus) binding protein (FKBP12) and subsequently it binds to a protein known as mTOR. Both compounds have an effector domain forming a composite surface with FKBP that interacts with the mammalian target of rapamycin, mTOR, as well as a binding domain that mediates the interaction with FKBP [64,66]. mTOR is an atypical serine/threonine protein kinase that belongs to the phosphoinositide 3-kinase PI3K-related kinase family and interacts with several proteins to form two distinct complexes named mTORC1 and mTORC2. mTORC1 responds to amino acids, stress, oxygen, energy and growth factors and is directly sensitive to sirolimus and everolimus. Cell growth is promoted by induction and inhibition of anabolic and

catabolic processes. mTORC1 also drives cell-cycle progression. In contrast, mTORC2 is insensitive to acute exposure of rapamycin, but chronic exposure can disrupt its structure. Moreover mTORC2 responds to growth factors and regulates cell survival and metabolism. mTORC 2 also regulates the cytoskeleton [67]. The mTORi-FKBP12-mTOR interaction causes dephosphorylation and inactivation of p70S6 kinase and which, when activated, stimulates the production of ribosomal components necessary for protein synthesis and cell-cycle progression. Cyclin dependent kinases (CDK) and cyclins are also inhibited, which are necessary to keep the cell cycle progress running. Consequently, sirolimus and everolimus inhibit T- and B-cell proliferation and differentiation and antibody production, as well as non-immune cell (fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells) proliferation [68–70]. When compared with sirolimus, the in vitro activity of everolimus is in general about two to three times lower; however, when administered orally, everolimus is at least as active in vivo as rapamycin [65].

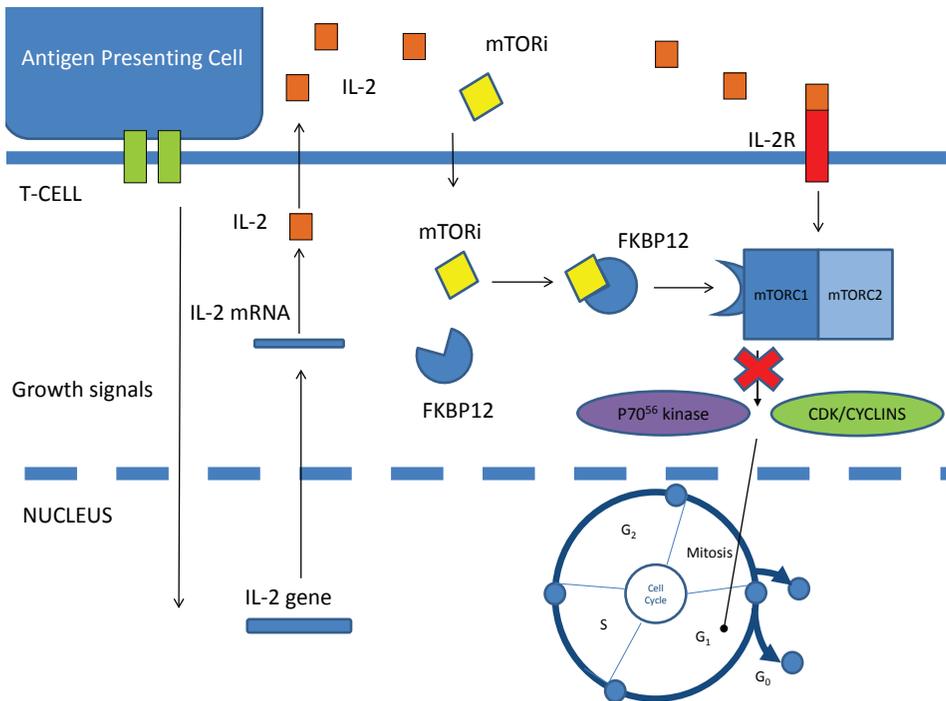


Figure 3: Simplified schematic representation of mTOR inhibitor mechanism of action. IL-2R, interleukin-2 receptor; IL-2, interleukin-2; mRNA, messenger ribonucleic acid; CDK, cyclin dependent kinase; FKBP12, FK506 (tacrolimus) binding protein; mTORi, mTOR inhibitor.

Side Effects

Sirolimus

The main and most common adverse effects attributed to sirolimus are anemia, thrombocytopenia and increase in triglyceride and cholesterol levels. Significant relationships were found between trough concentrations and the occurrence of thrombocytopenia ($<100 \times 10^9/L$), leukopenia ($<4 \times 10^9/L$) and hypertriglyceridemia (>750 mg/dL), but not hypercholesterolemia (>400 mg/dL). Toxic concentrations were established at >15 mg/L. Furthermore sirolimus has a narrow therapeutic window (≤ 5 $\mu\text{g/L}$) [19]. Hyperlipidemia occurs in about 40% of patients on sirolimus therapy. In a comparative study with azathioprine, increased fasting serum cholesterol and triglyceride concentration were observed, on average almost twice as high as in the azathioprine group. It is suggested that sirolimus inhibits the clearance of circulating, low, intermediate and very-low-density lipoproteins as well as their remnants [71,72]. Nevertheless only one patient discontinued the study because of hypertriglyceridemia and countermeasure therapy is often adequate [19]. Increased incidence of cardiovascular complications were not shown at phase III trials at one year after initiation [73]. Diarrhea incidence was also significantly higher than in the azathioprine group. Infections incidence including sepsis, cytomegalovirus, Epstein-Barr virus and Herpes zoster and lung infections were significantly higher in the 5 mg sirolimus group compared to the 2 mg sirolimus and the azathioprine group. The overall incidences of malignant disease besides lymphoma and lymphoproliferative disorders were similar in all treatment groups.

Everolimus

In a large (503 patients) multicenter study patients on a CNI free regimen of MPA and everolimus showed higher mean lipid concentrations, slightly increased urinary protein excretion, lower hemoglobin concentrations, also thrombocytopenia (6% vs 0%), aphthous stomatitis (15 vs 1%) and diarrhea (21 vs 8%) was reported more often compared to the CNI and MPA regimen. [11] A correlation was found between thrombocytopenia ($<100 \times 10^9/L$) with increasing everolimus AUC [49] and trends were observed for increased incidence of hypertriglyceridemia and hypercholesterolemia with increasing everolimus AUC. The incidence of leukopenia was not related to everolimus exposure. In a multicenter double blind, placebo controlled dose escalating phase I study, also dose dependent incidence of thrombocytopenia was found [74]. Notable reversible elevations of cholesterol were also observed at the 10 mg/day dose. Other changes in laboratory

evaluations, including triglycerides, were minor, reversible and did not appear to be dose dependent.

mTOR pneumonia

The use of mTOR inhibitors in renal transplantation is associated with many side effects as mentioned above: one of the potentially most severe being interstitial pneumonitis. Non-infectious interstitial pneumonitis is characterized by non-infectious, non-malignant and non-specific inflammatory infiltrates in combination with negative bacterial tests for blood and broncho alveolar lavage (BAL) [75,76]. Non-infectious pneumonitis is a class-related adverse effect of mTOR inhibitors. At the onset of this complication, patients present themselves with cough and/or dyspnea and/or hypoxemia. Sometimes systemic symptoms such as fever and fatigue are present. Pathology reveals non-specific interstitial pneumonitis, bronchiolitis obliterans organizing pneumonia, alveolar hemorrhage, desquamative interstitial pneumonia and vasculitis. The precise mechanism is unknown but one of the suggested mechanisms is a cell mediated autoimmune response after exposure of cryptic antigens or T-cell-mediated delayed-type hypersensitivity. Inhibitors of mTOR could also exert part of their action by limiting the destructive remodeling of lung structure. Over the years a number of case report were published concerning mTOR pneumonitis in transplantation [77–79]. The Incidence of pneumonia or pneumonitis with the usage of sirolimus (SRL) is about 1-10% [80]. The introduction of sirolimus led to an increased frequency of unexplained interstitial pneumonitis in renal transplant patients, which was later also observed in liver and heart transplant patients [81]. Because of its positive effect in cancer everolimus is currently also indicated for a number of oncological indications. This inflammatory disorder was also reported in everolimus-treated non-transplanted metastatic renal cell carcinoma patients at a frequency of 8% [82]. Another study reported a frequency of 9.9% with everolimus therapy [83]. So far, no clear patient-related or context-related risk factors have been identified. Many patients are asymptomatic despite presenting signs of the complication on radiography or high resolution tomography computer tomography (HRCT)[84]. The management of this mTOR pneumonitis depends on the grade of the side effect, Grade 1 with no clinical symptoms but a positive CT up to grade 4: Life threatening complications [85]. By identifying patients at risk for mTOR pneumonia before treatment patient could be excluded from mTOR therapy and switched to another immunosuppressive drug.

Therapeutic Drug Monitoring

Sirolimus

Sirolimus blood levels show good correlation with clinical outcomes and drug related toxicity [19,55]. Trough concentration (C_{trough}) AUC correlation seems reasonable [19], however others showed worse correlation [86]. AUC monitoring on the other hand is often laborious and patient unfriendly unless limited sampling formulas and models are used. In general Bayesian limited sampling models are less rigid than limited sampling formulas and are therefore more accurate. A number of these have been published [87–89] with sampling times 0,1 and 3 hours as the most accurate and with the least discomfort for the patient in a calcineurin inhibitor based regimen [89] using Bayesian estimation. AUC better reflects true exposure but whether AUC monitoring is superior to trough monitoring with respect to firm long-term endpoints has never been investigated. Whole blood concentration can be measured with a number of analytical techniques. Toxic concentrations were established at $>15 \mu\text{g/L}$ [19] and a therapeutic window has been proposed of 5-15 $\mu\text{g/L}$ or 6-12 $\mu\text{g/L}$ for calcineurin inhibitor included regimens and 10-20 $\mu\text{g/L}$ for regimens without calcineurin inhibitors [19,55,58]. Currently the most used techniques for sirolimus therapeutic drug monitoring (TDM) are liquid chromatography based techniques with or without mass spectrometry and immuno assay kits.

Everolimus

Since immunosuppression efficacy and occurrence and severity of side adverse effects are correlated with everolimus blood concentrations [25] TDM is also indicated. The recommended therapeutic range for everolimus evaluated as part of a calcineurin inhibitor regimen a number of studies is a trough of 3 to 8 $\mu\text{g/L}$ in renal transplant patients [90–93]. C_{trough} AUC correlation has not been intensively investigated in renal transplant patients. Everolimus target concentrations in a regimen without calcineurin inhibitors ranges from 6-10 $\mu\text{g/L}$ [10,11]. To date no limited sampling strategies have been developed for everolimus especially not in a cyclosporine free regimen. Currently the most used techniques for everolimus TDM are liquid chromatography based techniques with or without mass spectrometry and immuno assay kits.

Pharmacogenetics

A limitation of TDM is that during the critical period of the first days after transplantation the exposure cannot be influenced. Especially drugs with a long elimination half-life are at risk of under or overexposure because correcting them takes more time. For this reason pharmacogenetics could be of additional value to TDM, by differentiating in initial dose between genotype groups and subsequently decreasing the time to reach target concentration for all patients. However, whether this also leads to prolonged graft survival and lower incidence of acute rejection is not established. The mTOR inhibitors sirolimus and everolimus are metabolized by cytochrome P450 (CYP) enzymes CYP3A4, CYP3A5 and CYP2C8. Both compounds are also a substrate for the efflux pump P-glycoprotein (ABCB1). Genetic polymorphisms in genes encoding these enzymes could in theory explain a part of the variability in pharmacokinetics. Several single nucleotide polymorphisms (SNPs) have been identified in the genes encoding for CYP3A4, CYP3A5 and P-glycoprotein, including CYP3A4 -392A>G (rs2740574), CYP3A5 6986A>G (rs776746), ABCB1 3435C>T (rs1045642), ABCB1 1236C>T (rs1128503) and ABCB1 2677G>T/A (rs2032582) and some have been linked to pharmacokinetics of calcineurin inhibitors [94]. The most recognized clinically relevant single-nucleotide polymorphism (SNP) CYP3A5 A6986G has been linked in a number of studies to an increased tacrolimus clearance [95–97]. Initial dose adjustments have been proposed and are implemented in some transplantation centers. To date for CYP3A4 no conclusive results for candidate polymorphisms have been identified to optimize immunosuppressive therapy [98].

For mTOR inhibitors a limited number of pharmacogenetic studies have been published; *Le meur et al.* reported in a study of 47 patients that patients carrying at least one CYP3A5 SNP had significantly lower AUC/dose, C_{\max} /dose, C_{trough} /dose for sirolimus indicating a higher clearance [99]. In 22 renal transplant patients *Djebli et al.* found a 2 fold higher clearance for carriers of at least one CYP3A5*1 allele [89] compared to non-carriers. In another pharmacogenetic study of 149 renal transplant recipients the effect of CYP3A4 -392A>G (rs2740574), CYP3A5 6986A>G (rs776746), ABCB1 3435C>T (rs1045642), ABCB1 1236C>T (rs1128503) and ABCB1 2677G>T/A (rs2032582), on sirolimus pharmacokinetics was evaluated. CYP3A5 (around 1.5 fold higher compared to mutants) and CYP3A4 (almost 2 fold higher compared to mutants) genotype correlated significantly with concentration/dose ratio but variability within the genotype groups was considerable. This genotype effect however was only found in patients without a calcineurin inhibitor

[100]. Polymorphism in *ABCB1* did not correlate to different concentration dose ratio in all populations. Furthermore *Renders et al.* found a trend (not significant) for CYP3A5 expressors toward higher (2 fold) clearance in 20 renal transplant patients and no influence for *ABCB1* and *ABCC2* genotypes [101]. In contrast to the above mentioned findings *Mourad et al.* [102] found no association between adjusted trough concentrations and dose requirements and *CYP3A5* genotype in 58 renal transplant recipients.

For everolimus *Picard et al.* found no association between CYP3A5 polymorphism and everolimus pharmacokinetics in renal transplant patients [103]. Furthermore in vitro results supported this conclusion. The potential influence of polymorphisms in *CYP2C8* and *ABCB1* on everolimus pharmacokinetics is still unknown. More studies investigating the potential influence of polymorphisms in *CYP3A4*, *CYP3A5*, *CYP2C8* on pharmacokinetics and pharmacodynamics are needed to establish the potential influence and clinical relevancy.

The pregnane X receptor (PXR; NR1I2) is a member of the nuclear receptor (NR) superfamily. PXR is mainly associated with the cellular response to xenobiotics, including induction of enzymes involved in drug oxidation and conjugation, as well as induction of xenobiotic and endobiotic transporters [104]. These include the phase I enzymes cytochrome P450 (CYP) *CYP2C8* and *CYP3A4* and the transporters, multidrug resistance protein 1 (MDR1), MDR2, multidrug resistance-associated protein 2 (MRP2) and the organic anion transporter polypeptide 2 (OATP2) which are relevant for mTOR inhibitor metabolism, [105–107]. Polymorphism in genes coding for this receptor could be of interest for explaining variability in pharmacokinetics and dynamics [98].

Little is known about polymorphism genes coding for mTOR proteins and their effect on mTOR inhibitors pharmacodynamics. Recently *Woillard et al.* [108] examined candidate polymorphisms in mTOR, Raptor and p70S6 kinase and a number of other time-constant covariates and time varying covariates. They found an significant association in decrease of haemoglobin levels and an mTOR variant haplotype. However, critical questions were asked about the matching of the two study groups [109].

Conclusions

The macrolide immunosuppressant sirolimus and everolimus form a relatively new therapeutic group in renal transplantation and have shown their efficacy in recent trials. The

advantage of these compounds is the lack of nephrotoxicity compared to the calcineurin inhibitors cyclosporine and tacrolimus. In contrast to sirolimus everolimus is dosed twice daily because of its shorter half-life and is therefore easier to manage with therapeutic drug monitoring. Both drugs are metabolized by CYP3A4, CYP3A5 and CYP2C8 enzymes and are substrates for P-glycoprotein and share the same pharmacodynamics. The most important side effects of these are thrombocytopenia, leukopenia, hypercholesterolemia, diarrhea and although rare but potentially life threatening interstitial pneumonia. The narrow therapeutic window of mTOR inhibitors, together with high variability in pharmacokinetics, makes therapeutic drug monitoring essential for individualizing the dose and thereby prevent toxicity or rejection. Pharmacogenetics might play a role in further optimization of mTOR base immunosuppressive therapy.

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