Sirolimus, the first mTor inhibitor

J. A. Sánchez-Plumed*, M. González Molina, Á. Alonso*** and M. Arias******
Nephrology Service. La Fe* (Valencia), Carlos Haya** (Málaga), Juan Canalejo*** (La Coruña) and Marqués de Valdecilla**** (Santander) Hospitals.

Sirolimus (Rapamycin, Rapamune®) is a macrolide, product of the fermentation of an actinomycete, *Streptomyces hygroscopicus*, isolated (1975) from a soil sample in Rapa Nui (Easter Island), having a structure similar to tacrolimus (TaC) and to macrolide antibiotics[1-4]. Sirolimus (SRL) was initially studied as an antifungal agent[3] but it was observed in the first *in vitro* investigations that it exhibited potent immunosuppressant activity[5]. The first clinical trials with SRL in renal transplant patients were published in 1996[6]; «the study began on April 13, 1993», and since its approval for clinical use in the United States in 1999 and in Europe in 2000, it has aroused great interest in the transplant field due to its immunosuppressant potency and its antiproliferative and antitumor effects[7].

PHARMACOKINETICS

1. Absorption. SRL is quickly absorbed by oral route. Maximum blood concentration (t_{max}) is reached in healthy subjects with a single dose in approximately one hour and in renal transplant patients undergoing continued treatment, in 2 hours[1]. It has a low bioavailability, about 14%, due to the fact that it is metabolized by intestinal and hepatic cytochrome P-450 3A4 isoenzyme (P4503A) and eliminated, against the gradient, by intestinal glycoprotein P (P-gp)[8,9]. There is certain inter- and intrasubject variability in SRL pharmacokinetics and for that reason it is advisable to uniformly administer it with or without food, and when used in a solution it must be administered with water or orange juice. Sirolimus must not be administered with grapefruit juice because it alters isoenzyme CYP3A4-mediated metabolism[1]. Interindividual variations in SRL requirements are partially explained by the impact of polymorphisms. Patients carrying *CYP3A4*1B* and *CYP3A5*1* alleles require significantly larger doses to reach suitable blood concentrations[10].

2. Metabolism. SRL is a substrate of the CYP3A4 isoenzymes in the liver and small intestine[8]. It is metabolized by means of O-demethylation and/or hydroxylation[1], seven main blood metabolites having been identified, and some of them can also be detected in plasma, fecal and urine samples. SRL is the main component in blood and contributes to the immunosuppressant activity by more than 90%[1].

3. Excretion. 91.1% of SRL is eliminated through feces in about five days and 2.2% is eliminated through urine[1].

4. Pharmacokinetics in renal transplant recipients. All pharmacokinetic data support treatment (table I) with a single dose per day[1].

The mean whole blood/plasma ratio values of SRL were 36.4 and 36.8 after single and repeated oral doses, respectively, indicating that it is widely distributed in formed elements of the blood[1]. SRL in a human being mainly binds to serum albumin (97%), to α1 acid glycoprotein and to lipoproteins[1].

5. Pharmacokinetics in special populations:

a. Hepatic failure. SRL plasma clearance according to weight is lower (about 33%) in subjects with hepatic failure, therefore it is advisable to reduce the maintenance dose by one-third in these patients (1).

b. Renal failure. Patients with renal failure do not modify blood levels, so the dose need not be adjusted[1,11].

6. Levels. C_{max}, t_{max}, AUC and trough levels are dose-dependent. Trough levels of SRL correlate significantly with AUC ($r^2 = 0.95$). Patients treated with SRL (solution or tablets) with a loading dose for three

Table I. SRL pharmacokinetics (2 and 5 mg doses) in patients with renal transplant

	SRL doses	
	2 mg	5 mg
T_{max} (hours)	3.01 ± 2.40	1.84 ± 1.30
C_{max} (ng/ml)	12.2 ± 6.2	37.4 ± 21
C_{min} (ng/ml)	8.59 ± 4.01	17.3 ± 7.35
$CC_{plasmatic}$ (ml/hour/kg)	182 ± 72	221 ± 143

Correspondence: Dr. Jaime Sánchez-Plumed
Servicio de Nefrología
Hospital La Fe
Valencia
E-mail: jasanchezp@senefro.org

consecutive days reach stable blood trough levels 24 hours after the first maintenance dose[1].

When SRL in solution is administered simultaneously with Neoral cyclosporine (CsA), the C_{max} and AUC increase 116% and 230%, respectively. However if it is administered four hours after CsA, the C_{max} and AUC increase only 37 and 80%, respectively. The tablet preparation produces greater C_{max} and AUC increases. CsA C_{max} and AUC are not affected when SRL is administered in healthy volunteers in a single dose simultaneously with CsA or four hours later. However, CsA clearance decreases with continued doses of CsA and SRL and the dose must be reduced. Based on this data it was recommended for SRL to be administered four hours after CsA.

Renal transplant patients treated with SRL and TaC have greater renal dysfunction and arterial hypertension than those treated with TaC and mycophenolate mofetil (MMF), indicating that the combination enhances TaC nephrotoxicity. In fact, SRL blood concentrations increase with time after the transplant when it is associated with TaC. Therefore lower doses may be required to maintain therapeutic levels[12].

The SRL and MMF association increases mycophenolic acid (MPA) blood levels more than the CsA and MMF association does. There is interaction between both of them in the presence of CsA and the «first pass» intestinal absorption of MPA decreases because CsA inhibits P-gp activity and therefore the intestinal absorption of MPA[13,14].

ACTION MECHANISM

SRL has a different action mechanism from that of calcineurin inhibitors (CNI) and antimetabolites (fig. 1). It acts on the immune response by interfering with the transduction of the intracellular signal caused by the binding of interleukin-2 (IL-2) to its receptor, stopping the T-lymphocyte division cycle[4,15] from the G_1 to S phase. SRL binds to an intracellular receptor, binding protein FK (FKBP12), forming the SRL–FKBP12 complex inhibiting the mTOR enzyme (Rapamycin target) and therefore activation of the cyclin/CDK (cyclin-dependent kinases) and the phosphorylation of kinases (p70S6) necessary in regulating cell cycle progression[1,4,7,9-20]. *In vitro*, it inhibits growth of bone marrow cells, among them B lymphocytes, and the growth of other cell lines (mesenchymal and epithelial) in a variable proportion. This difference may be a reflection of the ability that certain cell lines have for substituting the loss of mTOR in the cell division cycle.

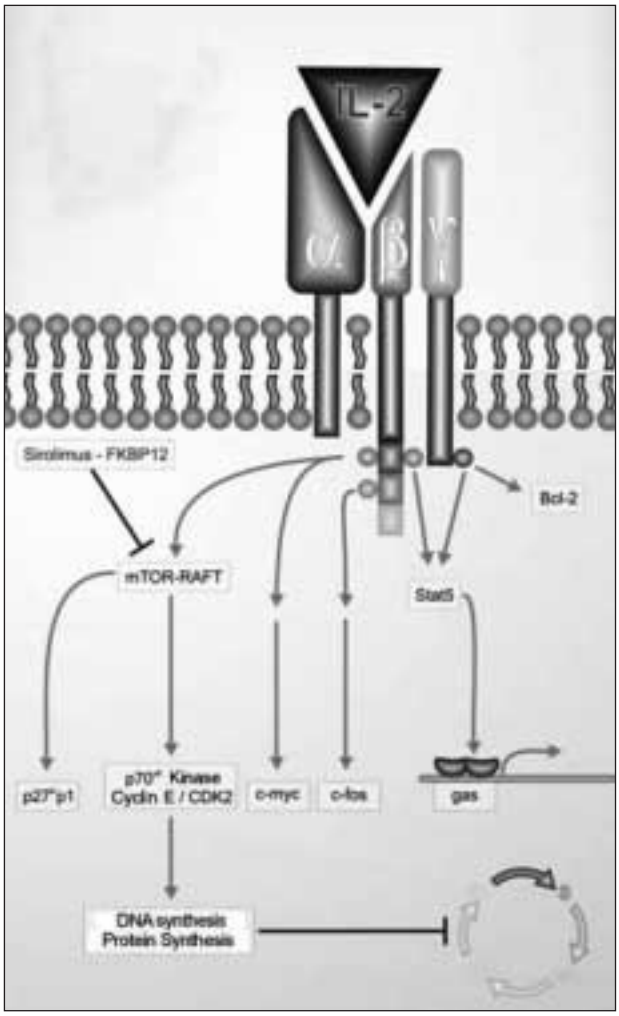

Fig. 1.—SRL inhibits the mTOR protein which induces activation of $p70^{S6K}$.

In summary, the immunosuppressant effect of SRL is a result of the inhibition of the activity and proliferation of T and B cells. It therefore acts on cell and humoral immunity[21]. The antiproliferative activity of SRL in vascular smooth muscle cells[22] and its ability to reduce intimal thickening in vascular damage models are due to its inhibiting the fibroblast growth factor (bFGF) and the platelet derivative[23]. The fact that SRL maintains apoptosis caused by IL-2 may be relevant in developing clinical strategies for achieving tolerance in the context of allogeneic transplantation[24,25]. In renal transplantation SRL (Table II) has proven to be a potent immunosuppressant capable of preventing acute rejection in different combinations with other immunosuppressants[26-34].

In addition to being a potent immunosuppressant, SRL has the following effects:

1. Antiproliferative. Vascular cell proliferation is an important component of chronic inflammatory response associated with atherosclerosis and occlusive vascular disease (restenosis after stent, transplant vasculopathy, etc.)[35]. The cells proliferating inside the atherosclerotic tissue are vascular smooth muscle cells, leukocytes and endothelial cells[36-38]. In an intimal lesion (more common in carotid arteries), monocytes and macrophages proliferate in a larger proportion and the smooth muscle cells in the media (more common in coronary arteries). The most important proliferation occurs in the first phase of the lesion[39]).

Cell proliferation and migration and the deposit of extracellular material contribute to the physiopathology of vascular obstructive disease (atherosclerosis, restenosis of coronary arteries after balloon angioplasty and stent implant, «chronic rejection

Table II. Incidence of acute rejection, graft survival and renal function in patients with renal transplant treated with SRL

Study	No. of patients	Acute R. %	G. survival (1 year) %	Plasmatic creatinine
301 (USA)[26]	719	(1 year)		(1 year)
CsA/Aza/P		31.1	93.8	160 mmol/l
CsA/SRL 2 mg/P		21.8	94.7	171 mmol/l
CsA/SRL 5 mg/P		14.6	92.7	133 mmol/l
302 (Global)[27]	576	(6 months)		(6 months)
CsA/placebo/P		41.5	87.7	155 mmol/l
CsA/SRL 2 mg/P		24.7	89.9	162 mmol/l
CsA/SRL 5 mg/P		19.2	90.9	150 mmol/l
207[28]	82	(1 year)		(1 year)
SRL, P, Aza	41	41	98	115 mmol/l
CsA, P, Aza	42	38	90	133 mmol/l
210[13]	161	(1 year)		(1 year)
SRL, MMF, P	81	27.4	92.5	128 mmol/l
CsA, MMF, P	80	18.4	89.5	143 mmol/l
[29,30]				
SRL, IL-2R, P, CsA		(1 year)		(1 year)
CsA, IL-2R, P	43	16	93	2 mg/dl
OKT3 o ATGAM,	21	52[a]	100	1.5 mg/dl
CsA, P		39[a]	78	1.5 mg/dl
212[31]	197	(1 year)		(1 year)
CsA, SRL 2 mg, P	97	18.6	92.8	1.82 mg/dl
1/2 CsA, SRL (10-20 ng/ml), CsA	100	22	95.0	1.38 mg/dl[a]
310[32]	525	(prerandom)	(all)	(1 year)
SRL, CsA, P		13.1	89	
Randomización	215			
SRL, CsA, P	215	4.2	95.8	158 mmol/l
SRL, P		9.8[a]	97.2	142 mmol/l [a]
Flechner SM[26]	61	(1 year)	(1 year)	(1 year)
Basi, SRL, MMF, P	31	6.4	96.7	1.32 mg/dl
Basi, CsA, MMF, P	30	16.6	95.4	1.78 mg/dl[a]
Sánchez-Plumed J[34]	977	6 months	6 months	(6 months)
TaC, P, SRL 0,5 mg	325	25.2	93	130 mmol/l
TaC, P, SRL 2 mg	325	15.7[a]	91	132 mmol/l
TaC, P, MMF 1 g	327	22.3	92	131 mmol/l

P: Prednisone. Aza: azathioprine. Basi: basiliximab. IL-2R: antibodies against the IL2 receptor. OKT3, monoclonal anti CD3 antibody. ATGAM, antithymocyte gamma globulin. [a]with statistical significance. The a of this table, which means.

in transplanted organs» transplant vasculopathy and vessel graft failures) resulting from the inflammation and the release of cytokines and growth factors. Therefore, treatment with antiproliferative agents such as SRL may be a therapeutic alternative in these disorders.

In an experimental hypercholesterolemia model, it has also been confirmed that SRL inhibits atherogenesis[40]. The action of SRL may be due to an increase in the $p27^{kip1}$ CDK inhibitor level and inhibition of pRb phosphorylation within the vessel wall, blocking the activity of the CDK/cyclin enzyme complexes, and therefore cell cycle progression[41]. The role of $p27^{kip1}$ in protecting against neointimal thickening has been proven in hypercholesterolemic apolipoprotein E-deficient mice in which genetic inactivation of one or of both $p27^{kip1}$ alleles accelerates atherogenesis[42]. However the inhibiting efficacy of the neointimal formation *in vivo* after mechanical damage was similar in mice with or without $p27^{kip1}$. Therefore, these findings confirm that SRL has antiproliferative and antimigratory activity, suggesting that it may contribute to controlling vascular manifestations of chronic rejection in organ transplantation, in controlling arterial restenosis after angioplasty and in post-transplantation cardiovascular complications[43]. SRL has proven to be effective in preventing coronary vascular lesion in heart transplantation[44].

SRL also contributes antiinflamatory and antifibrotic properties. At lower doses than those required for preventing acute rejection, it reduces interstitial inflammation and fibrosis associated with proteinuria in experimental membranous nephropathy and in rats it attenuates hepatic fibrosis produced in bile duct ligation[45,46].

2. Antitumor. Cancer is a frequent complication in organ transplant patients. The increase in the incidence thereof is mainly due to immunosuppressive treatment. Specifically it is the second cause of death in stable transplant recipients (19% of renal transplant patient deaths after the first year of evolution in La Fe Hospital). CsA favors tumor progression whereas SRL inhibits growth, *in vitro,* of tumor cell lines and exhibits antitumor activity in murine models. The immunosuppressant and antineoplastic effect of SRL may be due to a common mechanism. SRL inhibits mTOR synthesis involved in protein synthesis induced by stimulation with mitogens and in the cell division cycle progression when kinase p70S6 (fig. 1), a very important enzyme in regulating gene translation, is activated. Acute graft rejection is prevented due to mTOR inhibition since it interferes with T cell proliferation induced by IL-2 and at the same time blocks tumor generation and progression of the metastasis thereof due to inhibition of tumor cell proliferation. Every day new data surfaces favoring the antitumor effect of SRL.

a) Molecular basis of the antitumor effect:

- Induces apoptosis in B cell lymphoma B[47].
- In a murine model with a tumor inoculation of a human renal carcinoma cell line resembling the clinical course of renal carcinoma to a certain degree, SRL prevented pulmonary metastases development and prolonged survival of the recipients by blocking vascular endothelial growth factor VEGF and TGF-b1, whereas CsA favored pulmonary metastases development and reduced survival[48].
- It inhibits metastasis development by blocking angiogenesis[49]. This effect is due to the inhibition of VEGF formation.
- SRL and TGF-β cooperate in inhibiting the proliferation of nontransformed cells and tumor cells through inhibition of CDK2 activity[50].

b) Clinical data:

- Patients undergoing monotherapy (Table III) treated with SRL have a lower incidence of tumors than those who take it in association with CsA[51-54].
- SRL inhibits progression of Kaposi's sarcoma in renal transplant recipients while at the same time providing effective immunosuppression. The herpes 8 virus, which stimulates expression of endothelial cell Flk-1/KDR receptors, has been involved in the pathogenesis of Kaposi's sarcoma. Recent data further shows that Akt kinase of the mTOR stimulation pathway is activated in Kaposi's sarcoma and that Flk-1/KDR, Akt and p70S6 levels are high in Kaposi's sarcoma cells, probably due to the activation of VEGF receptors, a process that is inhibited by SRL[55-57].

3. Tolerance. Continued immunosuppressive treatment in organ transplant patients causes side effects that increase their morbimortality. Today it is known that the use of CNIs causes a progressive increase of arteriolar hyalinosis with vascular luminal diameter reduction, glomerulosclerosis and tubulointerstitial damage, such that after ten years 58.4% of the patients present severe chronic nephropathic lesions of the graft. As a result, a fundamental objective has been to find non-nephrotoxic drugs inducing tolerance. In this sense a study in

renal transplant patients treated with induction with thymoglobulin in order to perioperatively deplete T cells and to cause basic immunosuppression with SRL in monotherapy was designed. This treatment was well tolerated and the patients reached excellent renal function and a low acute rejection rate[58].

It has been experimentally demonstrated that a single dose of SRL (24 mg/kg on post-transplant day six) after administering antilymphocyte serum and donor-specific bone marrow transfusion, causes skin graft tolerance in mice. Specific tolerance is associated with chimerism, the level and duration of which is correlated with the bone marrow dose administered[59,60].

Trials combining Campath-1H and SRL have been performed, with promising preliminary results although with a relatively high rate (17%) of initial acute humoral rejection. T and B lymphocyte depletion occurs immediately after induction with Campath-1H and though it partially recovers, it is maintained with values that are less than the basal value one year after evolution. The most profound and prolonged depletion occurs in the CD4 lymphocytes. Monotherapy with SRL was well tolerated as was the CD4 lymphocyte depletion level, since no patient exhibited a system infection or malignant disease. After one year only one graft had been lost and no patients died[61].

Table III. Incidence of tumors in patients treated with SRL.

Multicentric study	Number of patients	Tumors
301 (USA) y 302 (GLOBAL) [26,27]	1295	
CsA/placebo/P	130	8,5%
CsA/Aza/P	161	5,5%
CsA/SRL 2 mg/P	511	5,0%
CsA/SRL 5 mg/P	493	8,4%
207[28]	82	
SRL, P, Aza	41	0%
CsA, P, Aza	42	4,7%
210[13]	161	
SRL, MMF, P	81	0%
CsA, MMF, P	80	5,0%
Kahan[54]		
Several protocols in which SRL is included	1.008	3%
310 (32)		
SRL, CsA, P	215	9,3%
SRL, CsA (3 meses), P	215	4,7%
Sánchez-Plumed J[34]	325	0%
TaC, P, SRL 0,5 mg	325	0,6%
TaC, P, SRL 2 mg	327	0%
TaC, P, MMF 1 g		

P: Prednisone, Aza: azathioprine

SIDE EFFECTS

1. Myelodepression. Thrombocytopenia is the most frequent complication in patients treated with SRL. It normally appears in the first four weeks of treatment and especially in cases with blood SRL levels above 16 ng/ml. The first episode is spontaneously resolved in 89% of the cases, but the dose must be reduced in 7% and temporarily suspended in 4% of the cases.

Leukopenia appears in a smaller proportion and its evolution shows similar characteristics to thrombopenia[62].

Anemia is normal in the initial period of treatment with SRL, both as an initial base immunosuppressant and after conversion by a CNI[63]. It correlates with the dose and blood concentration of the drug[27,28] and its incidence increases when associated to MMF[13,64]. Patients treated with SRL have a hematocrit 4.9% lower than those treated with other immunosuppressants[65-67]. Comparative analyses of the effects of SRL versus MMF in erythropoiesis in patients with renal transplants have shown that anemia is more frequent, severe and resistant in those treated with SRL[66] and its effect on the reduction of hemoglobin is independent from other known factors such as age, sex, infection and renal function damage[67]. The anemia is aregenerative, with high levels of ferritin and low levels of serum iron[68].

The mechanisms by which SRL produces anemia are:

- By inhibiting phosphatidylinositol-3-kinase activity. SRL blocks p70S6 kinase activity through this pathway and consequently erythroid cell line replication[69].
- By inhibiting insulin-like growth factor (IGF-1) by the p70S6 kinase pathway, involved in the regulation of post-renal transplant erythropoiesis[70].
- By inhibiting the 4E-BP1 (*eukaryotic initiation factor 4E-binding protein 1)* and consequently the erythroid cell division cycle[71]).

2. Delays in recovering graft function. SRL does not affect glomerular function[72] as CNIs do, but it

induces histological changes and tubular toxicity. In the renal failure by ischemia model[73], SRL inhibits the proliferation of renal tubular cells and favors their apoptosis. In renal transplant it increases the incidence of delayed graft function[74] and extends its recovery[75], suggesting that it exerts a toxic effect on the epithelial cells delaying their division, although this does not have a negative impact in graft function within a year nor in patient or graft survival. Furthermore, combined treatment with CNI may lead to an extension of cell damage and cell death (76). Consequently, the temporal association of severe ischemic damage of the graft with high doses of SRL does not seem to be ideal. Whether a low dose of SRL or the late introduction of its administration in patients treated with induction with poli- or monoclonal antibodies can improve these effects is yet to be determined.

2. Nephrotoxicity. Several studies have confirmed the deleterious effect of combining SRL with CsA, especially with standard doses[14,26]. Withdrawal of CsA in patients treated with both drugs produces a long term improvement in renal function[77] with favorable effects in graft histology[78]. It has also been observed that changing SRL for MMF in patients with renal function impairment treated with SRL/CsA is accompanied with improvement in renal function[79]. Experimental data suggests that combining SRL with TaC has less effect on the reduction of the glomerular filtrate and produces less fibrosis. It seems from the clinical data[80,81] that combining TaC and SRL is associated with a slight increase in creatinine, and it has been likewise reported that eliminating TaC is accompanied by a decrease in creatinine[33].

In short, SRL is an immunosuppressant with slight-moderate nephrotoxic properties regarding CNIs. The direct effects of SRL justify a certain degree of caution when used in induction in patients with risks for developing graft function delay or when administered for treating chronic graft nephropathy.

3. Proteinuria. Although a significantly greater incidence of proteinuria regarding the control group has not been observed in any of the studies performed with SRL in phases II and III, the occurrence of proteinuria in the nephrotic range in more than half the patients with chronic graft nephropathy after conversion from CsA to SRL has recently been notified[66,82]. The hemodynamic effect of CsA is indicated as the possible cause. This drug has a potent vasoconstriction effect and its withdrawal produces an increase in renal blood flow and in intraglomerular pressure which favor proteinuria[82]. On the other hand, the presence of focal segmental glomerulosclerosis has been observed in one third of the patients biopsied after occurrence of post-conversion proteinuria[66]. It is yet to be determined whether the sclerosis lesions in these patients are a consequence of the natural history of chronic graft nephropathy.

4. Glomerulonephritis. Four cases of glomerulonephritis, confirmed by biopsy, have been described in renal transplant recipients after conversion from a CNI to SRL. Renal function stabilization and regression of the proteinuria were observed after reintroducing the CNI, and in two patients the glomerulonephritis lesions disappeared[83].

5. Thrombotic microangiopathy (TMA). Treatment with SRL may induce TMA. Most cases described in patients treated with SRL and a CNI[27,84,85]. In a study of patients included in the USRDS the incidence, time of occurrence and risk factors of TMA were studied in 15,870 patients with renal transplant performed from January 1998 to July 2000 and SRL is identified as a risk factor for TMA. Given that the study starts from immunosuppression at the time of hospital release and does not indicate whether the patients had previously received a CNI and were converted to SRL, the results must be interpreted with caution[86]. Nevertheless, in two recent publications[87,88] three cases of TMA are described of TMA confirmed by renal biopsy in patients treated with SRL who received CNI. In our experience in the Hospital Universitario La Fe (*La Fe University Hospital*), one TMA confirmed by biopsy has been observed in a post-conversion from CsA to SRL case by chronic graft nephropathy, which improved after withdrawal of SRL.

6. Hypopotasemia e hypophosphatemia. SRL produces hypopotasemia and hypophosphatemia by tubular dysfunction. Hypopotasemia occurs more frequently during the first three months and is easily corrected with potassium supplements. Incidence varies from 8% to 27% of cases and is characterized by an increase in potassium excretion in the presence of hypopotasemia with a high transtabular gradient of potassium[89,90].

Patients treated with SRL have a reduction in tubular reabsorption of phosphates[90].

7. Edema and angioedema. Chronic edema defined as an edema with a duration period of more than one month, resistant to diuretics, and without a local, renal or cardiac cause is another of the complications of SRL. It is usually localized in lower limbs, but it may also occur in arms and eyelids; it is soft, non-inflammatory and may occur as an exacerbation of a previous edema. Its occurrence may be premature (developed during the

first month of treatment) or tardive (after two months of treatment). It can be asymmetric and in women it occurs in one of the upper limbs and in the homolateral breast. It usually occurs after a local trauma, an inflammatory disease or a local infection. It disappears or improves with suppression of SRL and only in exceptional cases does it persist after withdrawal of the drug[91-93]. The frequency of the chronic edema secondary to SRL is variable and there are authors who estimate it to be 55% of cases[91]. Among the mechanisms which may facilitate it there are inhibition of smooth muscle cell growth and reduction in endothelial growth factor production which alter blood pressure and local vascular permeability, facilitating occurrence of the edema. SRL also promotes the release of prostacyclins which produce vasodilation, which increases the possibilities of developing edema[47,94]. Two patients with psoriasis, treated with SRL, showed peripheral edema along with a generalized capillary leak syndrome[95].

The angioedema defined as a subcutaneous edema which resolves in less than four days is another complication due to SRL[94]. The frequency may be of up to 15% and the most frequent location is the face and in less proportion the tongue, lips, palate and neck. A high percentage of these patients took conversion enzyme inhibitors (ACEIs) or angiotensin II receptor antagonists (AIIRAs) and the cases not associated with this drug occurred after physical exercise or with ingestion of nuts or mango[91,96]. It is thought that the mechanism of the tongue edema is the result of an increase in prostaglandins which produces inadequate vasodilation with hyperpermeability of the vascular wall[99]. This effect may be enhanced with treatment with ACEI due to its action upon the kallikrein-kinin system[97].

8. Lymphocele. Patients treated with SRL have a greater incidence of lymphocele (8-20%) than those who receive other immunosuppressants[26,98]. Risk factors are obesity (BMI>30 kg/m^2) and the occurence of acute rejection episodes. The premature withdrawal of steroids (in five days) reduces the incidence of lymphocele (1.3%).

9. Healing of surgical wound. Treatment with SRL and obesity are independent risk factors for presenting surgical wound complications. Therefore, it is recommended to avoid treatment with SRL in the immediate postoperative period in obese patients[98].

10. Pneumonitis. Pulmonary toxicity associated to SRL represents a spectrum of clinical-pathological syndromes which are characterized by dyspnea, coughing, fever, asthenia and hemoptysis and histologically by the presence of bronchiolitis obliterans organizing pneumonia (BOOP), interstitial pneumonitis, focal fibrosis or alveolar hemorrhage. Although the usual clinical manifestations can present themselves after 12 months, it is usual for them to occur before 6 months or between 6-12 months after having initiated treatment with SRL, and they are expressed with dry cough, occasionally hemoptoic expectoration, dyspnea, asthenia, and occasionally fever and weight loss. The most common presentation varies from insidious to fulminant with fast clinical and radiological improvement and complete resolution in about three months after SRL withdrawal, although there are isolated cases of more delayed resolution. The frequency of its occurrence is not known exactly. Differential diagnosis must be performed with opportunistic infections, especially cytomegalovirus, pneumocystis carinii, legionella, mycoplasma pneumoniae, nocardia, cryptococcus, tuberculosis and adenovirus. Lymphocytes are found in the bronchoalveolar lavage (with T CD4 predominance), macrophages and siderophages.

Transbronchial biopsy shows features suggesting chronic interstitial pneumonitis and/or bronchiolitis obliterans organizing pneumonia (BOOP) with no evidence of tumoral cells, granulomas or infectious disease. Pulmonary function reveals a slight-moderate restriction pattern with a decrease in diffusion capacity.

The pathogenic mechanism of pulmonary toxicity induced by SRL is not known. It is thought that it is mediated by T cells and that the delayed hypersensitivity may be an alternative pathogenic mechanism. Repeated exposure to SRL in the lung leads to activation of the Th1 cells, Th1 cytokine release and macrophage and other inflammatory cell recruiting and activation. The occurrence of alveolitis (with moderate-important alveolar lymphocytosis) and the predominance of C4 cells (measured by flow cytometry) in the BAL support this hypothesis.

The treatment consists in the discontinuation or important reduction of the SLR dose, associated or not with corticoids. Although the possible benefits of high doses of steroids is uncertain, it has been verified that patients developing moderate-severe symptoms with a predominance of lymphocytic alveolitis or interstitial pneumonitis can improve with high doses of steroids[15,99-105].

11. Testosterone levels. Patients treated with SRL have lower testosterone levels than those receiving immunosuppressant treatment with other drugs and than the general population[106]. Its effect on the sexual function is not yet known.

TREATMENT OF NEPHROPATHY BY POLYOMAVIRUS BK (NBK)

Treatment with SRL is associated with a marked reduction of viral infections, essentially cytomegalovirus and VBK[54]. A favorable evolution of NBK in patients treated with TaC and MMF when converted to SRL and low doses of steroids has been recently published. The disappearance of decoy cells in urine, of viremia and an improvement of renal function took place without the patients developing any acute rejection episodes[107]. According to these authors, the disappearance of viremia BK after conversion to SRL-prednisone may be the result of several factors:

a) The restitution of immunity mediated by T cells after TaC and MMF suppression, which may prevent viral replication.
b) A decrease in virus permissibility in the absence of damage secondary to CNI chronic toxicity.
c) SRL inhibits neointimal and smooth muscle cell proliferation and can decrease fibrogenesis after the damage has been started.
d) It is possible that SRL has anti BK virus activity, as has been demonstrated with other viruses[108].

CONCLUSIONS

SRL is a liposoluble macrolide with a structure similar to that of TaC. It acts upon the immune response by interfering transduction of the intracellular signal produced by the union of IL-2 to its receptor, inducing a stop in the T cell cycle in the transition from phase G_1 to S. SRL facilitates apoptosis and acts synergistically with the blocking of the co-stimulating signal in reducing the number of alloreactive T lymphocytes.

C_{max}, t_{max}, AUC and trough levels of SRL are dose-dependent and their trough levels have a good correlation ($r^2 = 0.95$) with the AUC. When administered simultaneously with CsA, the C_{max} and AUC increase, but only slightly if taken four hours after the CsA. Current data has not shown interaction of TaC and SRL. When using the combination SRL and MMF, AMF levels are higher than in the case of CsA and MMF.

SRL, as well as its potent immunosuppressant action, presents an antiproliferative and antimigratory activity, and its vascular wall protective efficacy has been demonstrated in several animal models of catheter-induced damage and in clinical studies of coronary restenosis relapse prevention after dilation with stent and cardiac transplant arteriolopathy prevention. It has also been verified that it inhibits atherogenesis in an experimental model of hypercholesterolemia. This effect may be due to an increase in the CDK $p27^{kip1}$ inhibitor level and inhibition of pRb phosphorylation within the vessel wall, blocking CDK/cyclin enzymatic complex activity, preventing progression of the cell cycle.

SRL reduces the risk of cancer and the growth of established tumors, at the same time as it provides effective inmunosuppression.

REFERENCES

1. Rapamune^{R} (sirolimus) Ficha Técnica Autorizada (EMEA) Sep 03.
2. Kelly PA, Gruber SA, Behbod F, Kahan BD: Sirolimus, a new, potent immunosuppressive agent. *Pharmacotherapy* 17: 1148-1156, 1997.
3. Sehgal SN, Baker H, Vezina C: Rapamycin (AY-22.989), a new antifungal antibiotic. II. Fermentation, isolation and characterization. *J Antibiot* 28: 727-732, 1975.
4. Morris RE: Mechanisms of action of new immunosuppressive drugs. *Ther Drug Monit* 17: 564-569, 1995.
5. Martell RR, Klicius J, Galet S: Inhibition of the immune response by Rapamycin, a new antifungal antibiotic. *Can J Physiol Pharmacol* 55: 48-51, 1977.
6. Murgia MG, Jordan S, Kahan BD: The side effect profile of sirolimus: a phase I study in quiescent cyclosporine–prednisone-treated renal transplant patients. *Kidney Int* 49: 209-216, 1996.
7. Shih-Chieh J Cheuh and Bary D Kahan: Clinical application of sirolimus in renal transplantation: an update. *Transplant International* 18: 261-277, 2005.
8. Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, Christians U: Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Pharmacol Exp Ther* 285: 1104-1112, 1998.
9. Crowe A, Lemaire M: *In vitro* and *in situ* absorption of SDZ-RAD using a human intestinal cell line (Caco-2) and a single pass perfusion model in rats: comparison with Rapamycin. *Pharm Res* 15: 1666-1672, 1998.
10. Anglicheau D, Le Corre D, Lechaton S, Laurent-Puig P, Kreis H, Beaune P, Legendre C, Thervet E: Consequences of genetic polymorphisms for sirolimus requirements after renal. *Am J Transplant* 5: 595-603, 2005.
11. Zimmerman JJ, Kahan BD: Pharmacokinetics of sirolimus in stable renal transplant patients alter multiple oral dose administration. *J Clin Pharmacol* 37: 405-415, 1997.
12. McAlister VC, Mahalati K, Peltekian KM, Fraser A, Macdonald AS: A clinical pharmacokinetic study of tacrolimus and sirolimus combination immunosuppression comparing simultaneous to separated administration. *Therapeutic Drug Monitoring* 24 (3): 346, 2002.
13. Kreis H, Cisterne JM, Land W, Wramer L, Squifflet JP, Abramowicz D, Campistol JM, Morales JM, Grinyo JM, Mourad G, Berthoux FC, Brattstrom C, Lebranchu Y, Vialtel P: Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 9: 1252-1260, 2000.

14. Flechner SM, Goldfarb D, Modlin CH, Feng J, Krishnamurthi V, Mastonianni B, Savas K, Cook DJ, Novick AC: Kidney transplantation without calcineurin inhibitor drugs: a prospective, randomized trial of sirolimus *versus* cyclosporine. *Transplantation* 74: 1070-1076, 2002.
15. Sehgal SN, Camargo JS, Scarola JA, Maida BT: Rapamycin (sirolimus, rapamune). *Curr Opin Nephrol Hypertens* 4: 482-487, 1995.
16. Flanagan WM, Crabtree GR: Rapamycin inhibits p34cdc2 expression and arrests T lymphoyte proliferation at the G_1/S transition. *Ann NY Acad Sci* 696: 31-37, 1993.
17. Kuo CJ, Cheng J, Fiorentino DF, Flanagan WM, Blenis J, Crabtree GR: Rapamycin selectively inhibits interleukin-2 activation of p70 S6 protein kinase. *Nature* 358: 70-73, 1992. 11. Jayaraman T, Marks AR: Rapamycin-FKBP12 blocks proliferation, induces differentiation, and inhibits cdc2kinase activity in a myogenic cell line. *J Biol Chem* 268: 25385-25388, 1993.
18. Lorenz MC, Heitman J: TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. *J Biol Chem* 270: 27531-27537, 1995.
19. Zheng XF, Fiorentino D, Chen J, Crabtree GR, Schreiber SL: TOR kinase domains are required for two distinct functions, only one of wich is inhibited by Rapamycin. *Cell* 82: 121-130, 1995.
20. Morice WG, Brunn GJ, Wiederrecht G, Siekierka JJ, Abraham RT: Rapamycin induced inhibition of p34cdc2 kinase activation is associated with G1/S-phase, growth arrest in T lymphocytes. *J Biol Chem* 268: 3734-3738, 1993.
21. Kuypers DRJ: Benedit-risk assessment of sirolimus in renal transplantaron. *Drug Safety* 28: 153-181, 2005.
22. Marks AR: Rapamycin, signaling in vascular smooth muscle. *Transplant Proc* 35 Supl. 3ª: 231S-233S, 2003.
23. Stepkowski SM: Preclinical results of sirolimus treatment in transplant models. *Transplant Proc* 35 Supl. 3ª: 219S-226S, 2003.
24. Kirken RA, Wang YL: Molecular actions of sirolimus and mTOR. *Transplant Proc* 35 Supl. 3A: 227S-230S, 2003.
25. González J, Harris T, Childs G, Prystowsky MB: Rapamycin blocks IL-2 driven T cell cycle progresión while preserving T cell survival. *Blood Cells Mol Dis* 3: 572-585, 2001.
26. Kahan BD: Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomized multicentre study. The Rapamune US Study Group. *Lancet* 356: 194-202, 2000.
27. MacDonald AS, Rapamune Global Study Group: A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for preven of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 71: 271-280, 2001.
28. Groth CG, Bäckman L, Morales JM. Calne R, Kreis H, Lang P, Touraine JL, Claesson K, Campistol JM, Durand D, Wramner L, Brattstrom C, Charpentier B, for the Sirolimus European Renal Transplant Study Group. Sirolimus (Rapamycin)-based therapy n human renal transplantation. *Transplantation* 67: 1036-1042, 1999.
29. Hong JC, Kahan BD: Use of anti-CD25 monoclonal aintibody in combination with rapamycin to eliminate cyclosporine treatment during the induction phase of immunosuppression. *Transplantation* 68: 701-704, 1999.
30. Hong JC, Kahan BD: A calcineurin antagonist-free induction strategy for immunosuppression in cadaveric kidney transplant recipients at risk for delayed graft function. *Transplantation* 71: 1320-1328, 2001.
31. Gonwa TA, Hrick DE, Brinker K, Grinyó JM, Schena FP, for the Sirolimus renal Function Study Group: Improved renal function in sirolimus-treated renal transplant patients alter early cyclosporine elimination. *Transplantation* 74: 1560-1567, 2002.
32. Johnson RWG, Kreis H, Oberbauer R, Brattstrom C, Claesson K, Eris J: Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. *Transplantation* 72: 777-786, 2001.
33. Grinyó JM, Campistol JM, Paul J, García Martínez J, Arias M, Morales JM, Prats D, Cabrera J: Pilot randomized study of early tacrolimus withdrawal from a regimen with sirolimus plus tacrolimus in kidney transplanation. *Am J Transplant* 8: 1308-1314, 2004.
34. Sánchez-Plumed J, Pereira P, Lauzurica R, Arias M, Escuin F, Del Castillo D, Anaya F, Solá R, Ortuño J, Franco A, Valdés F, Capdevila L, Pallardó LM, Morales JM, González-Molina M: Multicéntrico. 8º Congreso Societat Catalana de Transplantament. Barcelona 8-9 de febrero 2005.
35. Dzau VJ, Braun-Dullaeus RC, Sedding DG: Vascular proliferation and atherosclerosis: new perspectives and therapeutics strategies. *Nature Med* 8: 1249-1256, 2002.
36. Katsuda S, Coltrera MD, Ross R, Gown AM: Human atherosclerosis. IV. Immunocytochemical analysis of activation and proliferation in lesions of young adults. *Am J Pathol* 142: 187-1793, 1993.
37. O'Brien ER, Alpers CE, Stewart DK, Ferguson M, Tran N, Gordon D, Benditt EP, Hinohara T, Simpson JB, Schwartz SM: Proliferation in primary and restenotic coronary atherectomy tissue. Implications for antiproliferative therapy. *Circ Res* 73: 223-231, 1993.
38. Rekhter MD, Gordon D: Active proliferation of different cells types including lymphocytes, in human atherosclerotic plaques. *Am J Pathol* 147: 668-677, 1995.
39. Veinot JP, Ma X, Jelley J, O'Brien ER: Preliminary clinical experience with the pullback atherectomy catheter and the study proliferation in coronary plaques. *Can J Cardiol* 14: 1457-1463, 1998.
40. Castro C, Campistol JM, Sancho D, Sánchez-Madrid F, Casals E, Andrés V: Rapamycin attenuates atherosclerosis induced by dietary cholesterol in apolipoprotein-deficient mice through a p27Kip1-independent pathway. *Atherosclerosis* 172 (1): 31-38, 2004.
41. Gallo R, Padurean A, Jayaraman T, Marx S, Roque M, Adelman S, Chesebro J, Fallon J, Fuster V, Marks A, Badimón JJ: Inhibition of intimal thickening afer balloon angioplasty in porcine coronary arteries by targeting regulators of the cell cycle. *Circulation* 99: 2164-2170, 1999.
42. Díez-Juan A, Andrés V: The growth suppressor p27(Kip1) protects against diet-induced atherosclerosis. *FASEB J.* 15: 1989-1995, 2001.
43. Morris RE, Cao W, Huang X, Gregory CR, Billingham ME, Rowan R, Shortouse RA: Rapamycin (sirolimus) inhibits vascular amooth-muscle cell DNA syntesis in vitro ans suppresses narrowing in arterial allogafts and in balloon-injured carotid arteries: evidence that rapamycin antagonizes growth factor action on inmune and noninmune cells. *Transplant Proc* 27: 430-431, 1995.
44. Keogh A, Richardson M, Ruygrok P, Spratt P, Galbraith A, O&rsquo, Driscoll G y cols.: Sirolimus in De Novo Heart Transplant Recipients Reduces Acute Rejection and Prevents Coronary Artery Disease at 2 Years. A Randomized Clinical Trial. *Circulation* 110 (17): 2694-2700, 2004.
45. Bonegio RG, Fuhro R, Wang Z, Valeri CR, Andry Carga, Salant DJ, Lieberthal W: Rapamycin ameliorates proteinuria-associates tubulointerstitial inflammation and fibrosis in experimental membranous nephropathy. *J Am Soc Nephrol* 16: 2063-2072, 2005.

46. Biecker E, De Gottardi A, Neef M, Unternahrer M, Schneider V, Ledermann, Sagesser H, Shaw S, Reichen J: Long-term treatment of bile Duch-ligated rats with rapamycin (sirolimus) significantly attenuates liver fibrosis: analysis of the underlying mechanisms. *J Pharmacol Exp Ther* 313, 052-061, 2005.
47. Muthukkumar S, Armes TM, Bondada S: Rapamycin, a potent immunosupprssive drug, causes programmed cell death in B lymphom cells. *Transplantation* 60: 364-370, 1995.
48. Luan FL, Ding R, Sharma VK, Chon WJ, Lagman M, Suthanthiran M: Rapamycin is an effective inhibitor of human renal cancer metastasis. *Kidney Int* 63: 917-926, 2003.
49. Guba M, Breitenbuch PV, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Antuber M, Jauch KW, Geissler E: Rapamycin inhibits primary and metastasic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nature Med* 8: 128-135, 2002.
50. Law BK, Chytil A, Dumont N, Hamilton EG, Waltner-Law ME, Aakre ME, Covington C, Moses HL: Rapamycin potentiates transforming growth factor beta-induced growth arrest in nontransformed, oncogene-transformed, and human cancer cells. *Mol Cell Biol* 22: 8184-8198, 2002.
51. Morales JM, Andrés A, Arias M, Campistol JM, García J, Grinyó JM, Gutiérrez Colón JA, Prats D: Enfermedad tumoral en receptores de trasplante renal que reciben sirolimus (SRL) (Rapamune) como terapia de mantenimiento: seguimiento a 2 años de 5 estudios multicéntricos. XXXII Congreso Nacional de la Sociedad Española de Nefrología. Bilbao, Abstract 264, 2002.
52. Charpentier B, Groth CG, Backman L, Morales JM, Calne R, Kreis H, Lang P, Touraine JL, Claesson Km Campistol JM, Durand D, Wramner L, Brattstrom C: Bicentre hospital experience with sirolimus-based therapy in human renal transplantation: Sirolimus Renal Transplant Study. Transplant Proc 35 (Supl.): 58S-61S, 2003.
53. Morales JM, Campistol JM, Kreis H, MouradG, Eris J, Schena FP, Grinyo JM, Nanni G, Andrés A, Castaing N, Brault Y, Burke JT: Sirolimus-based therapy with or without cyclosporine: long-term follow-up in renal transplant patients. *Transplant Proc* 37: 693-696, 2005.
54. Kahan BD, Knight R, Schoenberg L, Pobielski J, Kerman RH, Mahalati K, Yakupoglu Y, Aki FT, Katz S, Van Buren CT: Ten years of sirolimus therapy for human renal transplantation: The University of Texas at Houston experience. *Transplant Proc* 35: 25S-34S, 2003.
55. Stallone G, Schena A, Infante B, Di Paolo S, Loverre A, Maggio G, Ranieri E, Gesualdo L, Schena FP, Grandaliano G: Sirolimus for Kaposi´s sarcoma in renal-transplant recipients. *N Engl J Med* 352: 1371-1373, 2005.
56. Campistol JM, Gutiérrez Dalmau A, Torregrosa JV: Conversión to sirolimus: a successful treatment for post-transplantation kaposi's sarcoma. *Transplantation* 77: 760-762, 2004.
57. Zmonarski SC, Boratynska M, Rabczynski J, Kazimierczak K, Klinger M: Regression of Kaposi's sarcoma in renal graft recipients after conversion to sirolimus treatment. *Transplant Proc* 37: 964-966, 2005.
58. Swanson SJ, Hale DA, Mannon RB, Kleiner DE, Cendales LC, Chamberlain CE, Pollo SM, Harlan DM, Kira AD: Kidney transplantation with rabbit antithymocyte globulin induction and sirolimus monotherapy. *Lancet* 360: 1662-1664, 2002.
59. Hale DA, Gottschalk R, Maki T, Monaco AP: Determination o fan improved sirolimus (rapamycin)-based regimen for induction of allograft tolerante in mice treated with antilymphocyte serum and donor-specific bone marrow. *Transplantation* 65: 473-479, 1998.
60. Anam K, Akpinar E, Craighead N, Black AT, Hale DA: Targeted T-Cell Depletion or CD154 Blockade Generates Mixed Hemopoietic Chimerism and Donor-Specific Tolerance in Mice Treated with Sirolimus and Donor Bone Marrow. *Transplantation* 78: 1290-1298, 2004.
61. Knechtle SJ, Pirsch JD, Fechner JH, Becker BN, Friedl A, Colvin RB, Lebeck LK, Chin LT, Becker YT, Odorico JS, D'Alessandro AM, Kalayoglu M, Hamawy MM, Hu H, Bloom DD, Sollinger HW: *Am J Transplant* 3: 722-730, 2003.
62. Hong JC, Kahan BD: Sirolimus-induced thrombocytopenia and leukopenia in renal transplant recipients: risk factors, incidente, progresión, and management. *Transplantation* 69: 2085-2090, 2000.
63. Diekmann F, Budde K, Oppenheimer F, Fritsche L, Neumayer HH, Campistol JM: Predictors of success in conversión from calcineurin inhibitor to sirolimus in chronic allograft dysfunction. *Am J Transplant* 4: 1869-1875, 2004.
64. Winkelmayer WC, Kewalramani R, Rutstein M, Gabardi S, Vonvisger T, Chandraker A: Pharmacoepidemiology of anemia in kidney transplant recipients. *J Am Soc Nephrol* 15: 1347-1352, 2004.
65. Pareek R, Xiao S, Mian A, Kamra D, Lee J, Ahmed Z: Is the incidence of anemia higher in renal transplant patients on Mycophenolate Mofetil? *J Am Soc Nephrol*. Renal Week 2003. San Diego, November 12-17, 2003.
66. Augustine JJ, Knauss TC, Schulak JA, Bodziak KA, Siegel C, Hricik DE: Comparative effects of sirolimus and mycophenolate mofetil on erythropoiesis in kidney transplant patients. *Am J Transplant* 4: 2001-2006, 2004.
67. Morelon B, Kreis H: Sirolimus therapy without calcineurin inhibitors: necker hospital 8-year experience. *Transplant Proc* 35 Supl. 3A: 52S-57S, 2003.
68. Jaster R, Bittorf T, Klinken SP, Brock J: Inhibition of proliferation but not erythroid differentiation of J2E cells by rapamycin. *Bioch Pharmacol* 51: 1181-1185, 1996.
69. Morrone LF, Di Paolo S, Logoluso F, Schena A, Stallone G, Giorgino F, Schena FP: Interference of angiotensin-converting enzyme inhibitors on erythropoiesis in kidney transplant recipients: role of growth factors and cytokines. *Transplantation* 64: 913-918, 1997.
70. Panwalkar A, Verstovsek S, Giles FJ: Mamalian target of rapamycin inhibition as thrapy for hematologic malignancies. *Cancer* 100: 657-666, 2005.
71. Uddin S, Kottegoda S, Stigger D, Platanias LC, Wickrema A: Activation of the Akt/FKHRL1 patway mediates the antiapoptotic effects of erythropoietin in primary human erythroid progenitors. *Biochem Biophys Res Commun* 275: 16-19, 2000.
72. Thlivieris JA, Yatscoff RW: Effect of rapamycin on morphological and functional parameters in the kidney of the rabbit. *Transplantation* 59: 427-429, 1995.
73. Lieberthal W, Fuhro R, Andry C, Rennke H, Abernathy VE, Koh JS, Valeri R, Levine JS: Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol* 281: F693-F706, 2001.
74. Smith KD, Wrenshall LE, Nicosia RF, Pichler R, Marsh CL, Alpers CE, Polissar N, Davis CL: Delayed graft function and cast nephropathy associated with tacrolimus plus rapamycin use. *J Am Soc Nephrol* 14: 1037-1045, 2003.
75. McTaggart RA, Tomlanovich SJ, Bostrom A, Roberts JP, Feng S: Comparison of outcomes after delayed graft function: sirolimus-based versus other calcineuirn-inhibitor sparing induction immunosuppression regimens. *Transplantation* 78: 475-480, 2004.

76. Lawsin L, Light JA: Severe acute renal failure alter exposure to sirolimus-tacrolimus in two living donor kidney recipients. *Transplantation* 75: 157-160, 2003.
77. Kreis H, Oberbauer R, Campistol JM, Mathew T, Daloze P, Schena FP, Burke JT, Brault Y, Gioud-Paquet M, Scarola JA, Enhilan JF: Rapamune Maintance Regimen Trial. Long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal. *J Am Soc Nephrol* 15: 809-817, 2004.
78. Ruiz JC, Campistol JM, Grinyo JM, Mota A, Prats D, Gutiérrez JA, Henriques AC, Pinto JR, García J, Morales JM, Gómez JM, Arias M: Early cyclosporine a withdrawal in kidney-transplant recipients receiving sirolimus prevents progression of chronic pathologic allograft lesions. *Transplantation* 78:1312-1318, 2004.
79. Kaplan B, Schold J, Srinivas T, Comer K, Foley DP, Patton P, Howard, Meier-Kriesche HU: Effect of sirolimus withdrawal in patients with deteriorating renal function. *Am J Transplant* 4: 1709-1712, 2004.
80. Gionwa T, Méndez R, Yang HC, Weinstein S, Jensik S, Steinberg S for the Prograf Study Group: Randomized trial of tacrolimus in combination with sirolimus or mycophenolate mofetil in kidney transplantation: results at 6 months. *Transplantation* 75: 1213-1220, 2003.
81. Ciancio G, Burke GW, Gaynor JJ, Mattiazzi A, Roth D, Kupin W, Nicolas M, Ruiz P, Rosen A, Miller J: A randomized long-term trial of tacrolimus and sirolimus *versus* tacrolimus and mycophenolate mofetil *versus* cyclosporine (neural) and sirolimus in renal transplantation. II. Survival, function, and protocol compliance at 1 year. *Transplantation* 77: 252-258, 2004.
82. Taurina A, Campistol JM, Piera C, Campos B, Oppenheimer F: Cambios hemodinámicas glomerulares en pacientes trasplantados renales con nefropatía crónica del trasplante tras la conversión de ciclosporina a rapamicina. Congreso de la Sociedad Española de Nefrología Abstract 314, 2004.
83. Dittrich E, Schmaldienst S, Soleiman A, Horl WH, Pohanka E: Rapamycin-associated post-transplantation glomerulonephritis and its remission after reintroduction of calcineurin-inhibitor therapy. *Transpl Int* 17: 215-220, 2004.
84. Robson M, Cote I, Abbs I, Koffman G, Goldsmith D: Thrombotic microngiopathy with sirolimus-based immunosuppression potentiation of calcineurin-inhibitor-induced endotelial damage? *Am J Transplant* 3: 324-327, 2003.
85. Saikali JA, Troung LD, Suki WN: Sirolimus may promote thrombotic microangiopathy. *Am J Transplant* 3: 229-230, 2003.
86. Reynolds JC, Agodoa LY, Yuan CM, Abbott KC: Thrombotic microangiopathy alter renal transplantation in the United States. *Am J Kidney Dis* 42: 1058-1068, 2003.
87. Barone GW, Gurley BJ, Abul-Ezz SR, Gökden N: Sirolimus-induced thrombotic microangiopathy in a renal transplant recipient. *Am J Kidney Dis* 42: 202-206, 2003.
88. Crew RJ, Radhakrishnan J, Cohen DJ, Stern L, Goldstein M, Hardy M, D'Agati VD, Markowitz GS: De novo thrombotic microangiopathy following treatment with sirolimus: report of two cases. *Nephrol Dial Transplant* 20: 203-209, 2005.
89. Morales JM, Andrés A, Domínguez-Gil B, Sierra MP, Arenas J, Delgado M, Casal MC, Rodicio L: Tubular function in patients with hypokalemia induced by sirolimus after renal transplantation. *Transplant Proc* 35 (3 Supl.): 154S-156S, 2003.
90. Morales JM, Wramner L, Kreis H, Durand D, Campistol JM, Andrés A, Arenas J, Negre E, Burke JT, Groth CG: Sirolimus European Renal Transplant Study Group. Sirolimus does not exhibit nephrotoxicity compared to cyclosporine in renal transplant recipients. *Am J Transplant* 2(5): 436-42, 2002.
91. Mahé E, Morelon E, Lechaton S, Sang KH, Mansouri R, Ducasse MF, Mamzer-Bruneel MF, De Prost Y, Kreis H, Bodemer C: Cutaneous adverse events in renal transplant recipients receiving sirolimus-based therapy. *Transplantation* 79: 476-482, 2005.
92. Peddi VR, Jensil S, Pescovitz M, Pirsch J, Adler SH, Thistlethwaite JR, Vincenti F, Cohen DJ: An open-label, pilot study evaluating the safety and efficacy of converting from calcineurin inhibitors to sirolimus in established renal allograft recipients with moderate renal insufficiency. *Clin Transplant* 19: 130-136, 2005.
93. Mohaupt MG, Vogt B, Frey FJ: Sirolimus-associated eyelid edema in kidney transplant recipients. *Transplantation* 72: 162-164, 2001.
94. Moses JW, Leon MB, Popma JJ, Fitzgerald PJ, Holmes DR, O'Shaughnessy C, Caputo RP, Kereiakes DJ, Williams DO, Teirstein PS, Jaeger JL, Kuntz RE: SIRIUS Investigators. Sirolimus-eluting stents *versus* standard stents in patients with stenosis in a native coronary artery. *N Engl J Med* 349: 1315-1323, 2003.
95. Kaplan MJ, Ellis CN, Bata-Csorgo Z Kaplan RS, Endres JL, Fox DA: Systemic toxicity following administration of sirolimus (formerly rapamycin) for psoriasis: association of capillary leak syndrome with apoptosis of lesional lymphocytes. *Arch Dermatol* 135: 553-557, 1999.
96. Stallone G, Infante B, Di Paolo S, Schena A, Grandalino G, Gesualdo, Schena FP: Sirolimus and angiotensin-converting enzyme inhibitors together induce tongue oedema in renal transplant recipients. *Nephrol Dial Transplant* 19: 2906-2908, 2004.
97. Tschope C, Schultheiss HP, Walter T: Multiple interactions between the renin-angiotensin and the kallikrein-kinin systems: role of ACE inhibition and AT1 receptro blockade. *J Cardiovasc Pharmacol* 39: 478-487, 2002.
98. Dean PG, Lund WJ, Larson TS, Prieto M, Nyberg SL, Ishitani MB, Kremers WK, Stegall MD: Wound-healing complications after kidney transplantation: a prospective, randomized comparison of sirolimus and tacrolimus. *Transplantation* 77: 1555-1561, 2004.
99. Epler GR: Bronchiolitis obliterans organizing pneumonia: definition and clinical features. *Chest* 102: 2-6, 1992.
100. Singer SJ, Tiernan R, Sullivan EJ: Interstitial pneumonitis associated with sirolimus therapy in renal-transplant recipients. *N Engl J Med* 343: 1815-1816, 2000.
101. Morelon E, Stern M, Israel-Biet D, Correas JM, Danel Cl, Mamzer-Bruneel MF, Peraldi MN, Kreis H: Characteristics of sirolimus-associated interstitial pneumonitis in renal transplant patients. *Transplantation* 72: 787-790, 2001.
102. Digon BJ 3rd, Rother KI, Hirshberg B, Harlan DM: Sirolimus-induced interstitial pneumonitis in an islet transplant recipient. *Diabetes Care* 26: 3191, 2003.
103. Pham PT, Pham PC, Danovitch GM, Ross DJ, Gritsch HA, Kendrick EA, Singer J, Shah T, Wilkinson AH: Sirolimus-associated pulmonary toxicity. *Transplantation* 77: 1215-1220, 2004.
104. Haydar AA, Denton M, West A, Rees J, Goldsmith DJ: Sirolimus-induced pneumonitis: three cases and a review of the literature. *Am J Transplant* 4: 137-139, 2004.
105. Vlahakis Nicholas E, Rickman Otis B, Morgenthaler T: Sirolimus-associated diffuse alveolar hemorrhage. *Mayo Clinic Proc* 79: 541-545, 2004.
106. Fritsche L, Budde K, Dragun D, Einecke G, Diekmann F, Neumayer H-H: Testosterona concentrations and sirolimus in male renal transplant patients. *Am J Transplant* 4: 130-131, 2004.

107. Wali RK, Drachenberg C, Hirsch HH, Papadimitriou J, Nahar A, Mohanlal V, Brisco MA, Bartlett ST, Weir MR, Ramos E: BK virus-associated nephropathy in renal allograft recipients: rescue therapy by sirolimus-based immunosuppression. *Transplantation* 78: 1069-1073, 2004.

108. Roy J, Paquette JS, Fortín JF, Tremblay MJ: The immunosuppressant rapamycin represses human immunodeficiency virus type I replication. *Antimicrob Agents Chemother* 46: 3447-3455, 2002.