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Rapamycin rescue therapy in patients after kidney transplantation: first clinical experience

Thomas Weber,[1] Dietmar Abendroth[2] and Hubertus Schelzig[3]

1 Department of Thoracic Surgery, Inselspital Bern, University Hospital, Bern, Switzerland
2 Department of Visceral and Transplantation Surgery, University Hospital, Ulm, Germany
3 Department of Thoracic and Vascular Surgery, University Hospital, Ulm, Germany

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Correspondence
Dr Thomas Weber, Department of Thoracic Surgery, University Hospital, Freiburgstrasse, CH-3010 Bern, Switzerland. Tel.: +41-31-6320470; fax: +41-31-6322327; e-mail: thomas.weber@insel.ch

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Summary

This study was aimed at analysing rapamycin (RAPA) rescue therapy with calcineurin inhibitor (CNI) withdrawal in renal transplant patients primarily presenting with CNI-nephrotoxicity (CNI-Neph), chronic allograft nephropathy (CAN) without [CAN(a)] and with histological changes suggestive of chronic rejection [CAN(b)]. In 36 patient with CNI-Neph ($n = 6$), CAN(b) ($n = 21$), CAN(a) ($n = 7$), and others ($n = 2$) RAPA therapy was started 4.4–115 months (median 30.6 months) after renal transplantation. During a follow up of 3–33 months (median 19 months) parameters of kidney function were recorded. Three patients on haemodialysis did not show any recovery of graft function. Of the remaining 33 patients renal function improved in 22 (66.7%), was stable in three (9%) but deteriorated in eight (24%) patients, of whom seven (21%) required haemodialysis thereafter. Success rate of RAPA therapy differed with respect to the histological diagnosis: 70% in CAN(b), 80% in CNI-Neph and 33% in CAN(a). Furthermore, in patients with creatinine levels above 400 μM ($n = 6$) graft function rarely improved ($n = 2$, 33%). The RAPA rescue therapy with CNI withdrawal appears promising in a special cohort of patients with chronic renal allograft dysfunction even late after transplantation.

Introduction

Although calcineurin inhibitor (CNI)-based immunosuppression has dramatically increased the 1 year kidney graft survival to more than 80% during recent decades [1], CNI-related nephrotoxicity (CNI-Neph) limits the long-term outcome of renal transplants at the same time [2]. Early after transplantation CNIs may cause vasoconstriction of renal arterioles and arteries, leading to altered renal haemodynamics and a reduced glomerular filtration rate [3]. In biopsy histology a vacuolation of endothelial and smooth muscle cells can be observed [4]. The nodular appearance of arteriolar hyaline thickening has been recognized as a particular manifestation of CNI-Neph by the Banff 97 working classification [5].

Chronic rejection primarily denotes to alloantigen-dependent processes caused by cell-mediated and/or antibody-mediated immunity that lead to structural and finally functional deterioration of an allograft [6,7]. Infiltrating T cells, other mononuclear cells and/or antibodies directed against human leucocyte antigen (HLA) or epithelial and endothelial cell antigens can be found [6–8]. At the same time, the clinical diagnosis of chronic rejection requires a significant increase of plasma creatinine levels in at least two measurements, taken 3 months apart, with the first assessment made at least 3 month after transplantation.

Beside chronic rejection, possibly modified by an additional antigen stimulation, a series of nonimmune disturbances or their sequels persist or come into play (transfer of donor vascular lesions, acute tubular necrosis, hypertension, dyslipidaemia) [7–10]. Their common and most important consequence is tissue ischaemia [10,11]. Unlike in the early period, the metabolic disturbances assume an

increasing significance, especially the 'atherogenic' increase in low-density lipoproteins [9]. The differentiation of individual pathogenic factors – as well as the role of innate immunity – is frequently difficult or impossible, so that the general descriptive term 'chronic allograft nephropathy' (CAN) has been coined to label the late morphological changes [12]. The Banff 97 classification uses the term chronic/sclerosing allograft nephropathy and defines three different grades based on the severity of interstitial fibrosis and tubular atrophy. Each grade is further categorized according the absence (a) or presence (b) of the typical vascular changes of chronic rejection [5].

Thus, although CNI-Neph, CAN(a) and CAN(b) share the final and common feature of chronic renal allograft dysfunction, which is the most prevalent cause of renal graft loss after the first year [13], they can be differentiated by few but characteristic changes in biopsy histology [4,5]. From a clinical point of view this may become increasingly important, as new immunosuppressive agents with different mechanisms of action have become available.

In this regard rapamycin (RAPA; sirolimus) may emerge as an interesting alternative to CNIs. Beside its immunosuppressive capability RAPA bears high antiproliferative and antitumour properties [14]. RAPA effectively prevents myointimal proliferation in models of coronary angioplasty in the pig [15] and allograft vascular disease in nonhuman primates [16]. At the same time RAPA exerts no nephrotoxic side-effects [17]. As a result former studies have documented decreasing creatinine levels and increasing glomerular filtration rates after CNI withdrawal in patients with RAPA maintenance therapy [18,19]. Considering those positive effects of RAPA on chronic proliferative processes and kidney functions we have studied RAPA as rescue therapy in patients with deteriorating kidney allograft function.

Patients and method

Between June of 2000 and September of 2002, 36 renal transplant patients (aged 19–68 years) were converted from cyclosporin (CsA; Neoral® (Novartis, Basel, Switzerland), $n = 23$) or tacrolimus (FK506; Prograf® (Fujisawa, Munich, Germany), $n = 13$) based immunosuppression to RAPA (Rapamune® Wyeth, Muenster, Germany). Five patients were on CsA or Tac monotherapy, all others had a combination with mycophenolate mofetil (MMF; Cellcept® Roche, Reinach, Switzerland). The female/male ratio was 17/19, 29 patients had a first, six patients a second and one patient a third transplant. The mean mismatch for HLA A/B/DR was 3.2 ± 0.8. The time between kidney transplantation and start of RAPA rescue therapy ranged between 4.4 and 115 months (mean 42 ± 29, median 30.6 months), the follow up after conversion to RAPA ranged between 3 and 33 months (mean 17 ± 9, median 19 months).

In 34 patients, a graft biopsy was percutaneously taken before immunosuppressive therapy was switched to RAPA. For the purpose of this study and in accordance with the Banff classification the following histological criteria were applied [5]. CAN(a): interstitial fibrosis, tubular atrophy and/or loss, glomerulopathy and mesangial matrix increase (grades 1–3a); CAN(b): interstitial fibrosis, tubular atrophy and/or loss together with typical vascular lesions and mononuclear infiltrates (grades 1–3b); CNI-Nep: hyaline changes particularly in the afferent arterioles of the glomerulus and vacuolation of tubular epithelial cells.

On the basis of this differentiation there were 21 patients with CAN(b), six with CNI-Neph and seven with CAN(a). Two patients were converted to RAPA because of insufficient CsA resorption with increasing creatinine levels but without having histological examinations.

Conversion to RAPA was performed within 3 weeks. RAPA was started with a loading dose of 5–8 mg orally once a day for the first 2 days. The following day RAPA trough levels were measured for the first time. Thereafter, oral doses were concentration-controlled to keep 24 h trough levels at 8–12 ng/ml at any time. CsA and FK506 doses were reduced by 33% starting 1 week after conversion to RAPA, thereafter every week again by 33%. Concomitant immunosuppression with MMF was usually continued at a dose of 250 mg twice a day. Corticosteroids and antithymocyte globulin were not given at any time.

After conversion to RAPA following parameters were prospectively measured: plasma creatinine (μM), plasma urea (mM), haemoglobin (g/dl), leucocytes (Giga/l), thrombocytes (Giga/l), plasma cholesterol (mM) and triglycerides (mM).

Statistics

Data were tested for normal distribution using the Kolmogorov–Smirnov test (Lilliefors), placing the confidence level at 95%. As normal distribution was not always confirmed significance of difference was assessed using the Wilcoxon signed rank test. A probability value of $P < 0.05$ was regarded as being statistically significant.

Results

Of all 36 patients entering this study, three already required constant haemodialysis when RAPA therapy was started. Histological diagnosis was CAN(a), CAN(b) and CNI-Neph in one case each. In none of those three patients any improvements in kidney allograft function was observed.

However of the 33 patients, who did not require haemodialysis at time of RAPA conversion, 22 (66.7%) showed significant improvements in graft function (Fig. 1). Creatinine and urea levels decreased between 22–25% and 28–42%, respectively, during the first year after conversion ($P < 0.05$). A positive response to RAPA with decreasing serum creatinine levels was observed within 2 weeks after conversion in four patients (18%), within 4 weeks in eight patients (36%), within 8 weeks in seven patients (32%) and within 12 weeks in three patients (14%). Three patients (9%) showed no considerable changes of graft function. Creatinine levels were 205, 252 and 412 μM before and 210, 250 and 377 μM after 1 year of conversion.

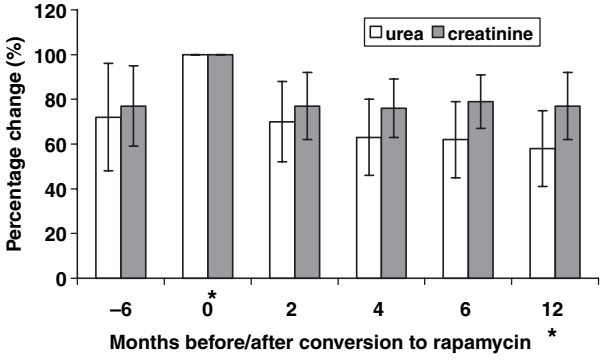

Figure 1 Changes of creatinine and urea in 22 patients with improving kidney graft function during the first year of rapamycin rescue therapy (creatinine/urea levels at time of conversion* were considered as 100%, mean ± SD).

In eight patients (24%) a further deterioration of kidney allograft function developed, of whom seven patients (21%) went on constant dialysis during the first 12 month after conversion to RAPA.

To characterize patients possibly responding to RAPA rescue therapy, patients were grouped according to their creatinine levels before conversion. As depicted in Table 1, the success rate of RAPA was considerably better in patients with preconversion creatinine levels below 400 μM (groups I and II) when compared to patients with a creatinine of more than 400 μM (group III). The absolute creatinine changes (preconversion/12 month) in patients responding to RAPA therapy were in group I: 210 ± 25/172 ± 34 μM, group II: 336 ± 41/258 ± 52 μM and in group III: 435 ± 5/332 ± 53 μM. From the six patients of group III only two patients with chronic rejection, in one case accompanied with cytomegalovirus (CMV) infection, responded to RAPA rescue therapy.

Table 2 shows the influence of the histological diagnosis on the response rate to RAPA rescue therapy. Significantly improved kidney functions were observed in 70% and 80% of patients with CAN(b) and CNI-Neph, respectively, whereas only 33.3% of patients with CAN(a) showed a positive response. Of all patients not responding to RAPA a considerable proportion later required continuous haemodialysis (CAN(b) 25%, CAN(a) 33.3%). Both patients, in which insufficient CsA resorption was diagnosed, had improving kidney functions after conversion to RAPA.

Two year results of RAPA rescue therapy were available for 14 patients with formerly improving kidney function at 12 months. As depicted in Fig. 2 creatinine levels

Table 1. Influence of creatinine levels before conversion to rapamycin rescue therapy on subsequent kidney graft function.

			Kidney graft function			
	Preconversion creatinine (μm)	*n*	Improved (%)	No change (%)	Worse (%)	Dialysis (%)
Group I	<265 (3 mg/dl)	9	7 (77.8)	2 (22.2)	0	
Group II	265–400 (3–4.55 mg/dl)	18	13 (72.2)	0	5 (27.7) ➡	4 (22.2)
Group III	400 (4.55 mg/dl)	6	2 (33.3)	1 (16.7)	3 (50) ➡	3 (50)

Table 2. Correlation between histological or clinical diagnosis and the subsequent development of kidney graft function in patients with rapamycin rescue therapy.

		Kidney graft function			
	n	Improved (%)	No change (%)	Worse (%)	Dialysis
Chronic allograft nephropathy [CAN(b)] suggestive of chronic rejection	20	14 (70)	0	6 (30) ➡	5
Calcineurin inhibitor-nephropathy (CNI-Neph)	5	4 (80)	1		
Chronic allograft nephropathy [CAN(a)] with no signs of chronic rejection	6	2 (33.3)	2 (33.3)	2 (33.3) ➡	2
Others*	2	2			

*Insufficient CsA resorption with increasing creatinine levels.

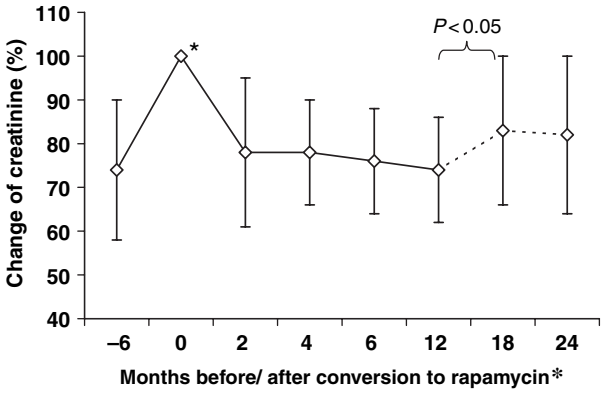

Figure 2 Two year results (change of creatinine) of rapamycin rescue therapy in 14 patients, who had improving kidney graft functions at 1 year (creatinine levels at time of conversion* were considered as 100%, mean ± SD).

significantly increased between 12 and 18 months after conversion.

Side-effects

A severe thrombocytopenia (<150 Giga/l) was only observed in one (3%) and leucopoenia (<4.4 Giga/l) in six patients (18%). Despite erythropoietin treatment in 22 patients (67%), 10 patients had haemoglobin levels between 5.7 and 9.9 g/dl during the first year. In addition, hypertriglyceridaemia (>2.3 mM) and hypercholesteremia (>5.2 mM) was observed in 50% and 82% of our patients, respectively, despite statin therapy.

Discussion

The safety and efficacy of RAPA maintenance therapy in kidney transplant patient has been documented by the largest randomized CsA withdrawal study conducted in Europe, Australia and Canada [19]. Patients on RAPA/steroid immunosuppression with or without CsA had identical graft and patient survival after 12 months. However, creatinine levels and glomerular filtration rate significantly improved, when CsA had been withdrawn 3 months after transplantation [19]. Recently, the 2 year results of this trial were published [20]. Whereas creatinine further increased in patients still receiving CsA it further decreased in patients treated with RAPA without CsA.

Our study design differed with respect to the time of CNI withdrawal. All patients with CNI-Neph, CAN(a) and CAN(b) were switched on a CsA-free protocol at different points of time after transplantation (median 30.6 months, range: 4.4–115). Nevertheless, kidney graft function significantly improved in 80% of patients with CNI-Neph. Although this subgroup of patients was rather small, our favourable results are supported by others [21–23].

To our knowledge, this is the first clinical report showing beneficial effects of RAPA on kidney graft function in patients with histological changes suggestive of chronic rejection [CAN(b)]. During the first 12 months after conversion to RAPA 70% of those patients had significantly improving creatinine levels. The interpretation of these findings in CAN(b) is difficult, since CNIs are still supposed to have a higher immunosuppressive potency in the prevention of rejection episodes. However, our results in CAN(b) patients may implicate that secondary proliferative processes within the kidney graft may play an increasingly important role in chronic rejection, which may respond to RAPA therapy. This is supported by studies in animal models for chronic allograft rejection. Poston *et al.* reported, that even delayed RAPA treatment, starting 60 days after transplantation, reversed chronic graft vascular disease in rats [24]. Similar results were reported by Ikonen *et al.* in nonhuman primates [16]. Recently Jolicoeur *et al.* demonstrated a specific inhibition of vascular fibrous intimal thickening, allograft glomerulopathy and interstitial fibrosis in rat renal allografts, when RAPA in combination with MMF was started 4 weeks after transplantation [25].

A clear estimation to which extend CNIs and proliferative processes individually contribute to chronic allograft dysfunction, is frequently elusive. However, our good results in those patients with a histologically assigned CNI-Nep and CAN(b) indicate that CNI withdrawal together with the antiproliferative properties of RAPA positively influences renal allograft function in a special cohort of patients.

In contrast, the success rate of RAPA rescue therapy in patients with histologically diagnosed CAN(a) was low (33.3%). Recently Saunders *et al.* found absolutely no beneficial effects of RAPA in 40 patients with CAN [26]. However in their study, patients received only a 40% reduction of CsA doses and low dose RAPA therapy (2 mg/day). Fixed dose regimens of RAPA may not be sufficient due to the high inter- and intrapatient variability of trough drug concentrations of RAPA.

Referring to Kreis and Ponticelli, the term CAN denotes to a chronic renal pathology, which can be caused by one or any combination of the following factors: chronic rejection (alloantigen-dependent immune process) and alloantigen-independent immune (e.g. reperfusion-ischaemic injury, CMV-related changes) and nonimmune processes (e.g. CNI toxicity or severe, acute tubular necrosis [27]). The interpretation of this wide pool of aetiologies possibly attributing to CAN is difficult but may point to the fact that particularly CAN(a) might represent the advanced

and finally common pathology of all entities chronically affecting renal allografts. At this stage, morphological changes may not be reversible. This point of view would explain the low success rates of RAPA rescue therapy in patients with an assigned CAN(a) histopathology and the inverse correlation between creatinine levels and the response to RAPA rescue therapy in our study. The higher the creatinine levels before conversion (400 μM) the lower the percentage of patients (33.3%) showing any significant improvements in kidney function. Moreover, patients with totally deteriorated kidney functions, already necessitating haemodialysis before RAPA therapy was started, did not improve, independent of the assigned pathology of CNI-Neph, CAN(a) or CAN(b).

Despite the positive effects of RAPA on kidney graft function in a considerable proportion of patients over a period of 12 months, creatinine levels deteriorated again after 18 and 24 months. On the basis of our actual analysis we can only speculate on this observation, which in our view might be related to the patient's compliance. However, other immunological and nonimmunological processes of chronic allograft damage have to be considered as well, which are not sufficiently controlled by RAPA therapy. Therefore, the long time impact of RAPA rescue therapy in patients with chronic allograft dysfunction requires further attention.

In conclusion, a selected group of renal transplant patients may profit from RAPA rescue therapy over a period of about 12 months. The results in patients with CNI-Neph and CAN(b) are promising. In contrary, kidney allograft function seldom improves in patients with CAN(a) and creatinine levels of more than 400 μM and, in our experience, never in patients already requiring chronic dialysis before conversion.

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