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ORIGINAL ARTICLE

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Sirolimus and mycophenolate mofetil after liver transplantation

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Abstract Since the approval of sirolimus (SRL) as an immunosuppressive agent in renal transplantation, several liver transplant centres have introduced this agent to the immunosuppression regimen. We present here a retrospective follow-up study of late conversion to sirolimus and mycophenolate mofetil (MMF) as immunosuppressive agents after liver transplantation (LTX). From July 2001 to March 2002, seven liver transplant recipients (three female, 59 (41–66) years old) were enrolled in this study. Indications for liver transplantation were hepatitis B and/or hepatitis C (three), alcohol-induced cirrhosis (three) and Wilson's syndrome (hepatolenticular degeneration) (one). LTX was performed by standard (four) or piggy-back (three) technique. The switch to SRL was performed 62 (37–118) months after LTX; the reasons for the switch from cyclosporine or tacrolimus to SRL were renal (six) or neurological (one) impairment. As immunosuppressive therapy, SRL at trough levels of 4–10 ng/ml and MMF at trough levels of approximately 1 μg/ml were administered. Mean follow-up time under SRL per patient was 137 (26–258 days). Patient and graft survival was 100% during SRL therapy, and there were neither rejection episodes nor infections. Renal function improved in five of the six patients (83.3%) whom we had switched to SRL due to renal impairment. In the patient whom we switched to SRL due to neurological impairment, the neurological symptoms abated, and renal function improved. Side effects (hypertriglyceridaemia, hypercholesterolaemia, exanthema) became manifest in three patients (42.8%). Cessation of therapy due to side effects was necessary in two patients (exanthema: one, hypertriglyceridaemia: one). One patient refused to continue the therapy with SRL because he wanted tablets, and we only had SRL in fluid form. The data of our study suggest that SRL is a potent immunosuppressive agent of potential benefit in clinical LTX. SRL in combination with MMF provided sufficient immunosuppression of liver allografts in the late course after LTX. Side effects were reversible with dose reduction or cessation of therapy. We can thus conclude that SRL might offer an immunosuppressive therapy for patients with renal or neurological impairment after LTX.

Keywords Liver transplantation · Immunosuppression · Sirolimus · Nephrotoxicity

Introduction

The standard of immunosuppression in transplant recipients involves the use of calcineurin inhibitors such as cyclosporin A (CsA) and tacrolimus (TAC). These agents have a significant impact on the increasing survival rates in patients following transplantation; however, both CsA and TAC are associated with significant nephrotoxic side effects in the short and long term [12].

Despite huge advances in liver transplantation, reaching 1-year survival rates of approximately 90% in most centres, many long-term survivors face a considerable risk of renal dysfunction due to the conventional immunosuppressive agents. In many cases this leads to deterioration of renal function, with high morbidity and mortality rates [4]. The search for alternative therapies against hepatic allograft rejection without producing nephrotoxicity continues. New non-nephrotoxic agents such as sirolimus (SRL, rapamycin) and mycophenolate mofetil (MMF) appear promising, but more data are needed to support their use [4].

SRL, a macrocyclic lactone, isolated from *Streptomyces hygroscopicus*, is a new immunosuppressive agent. SRL has potent immunosuppressive properties that are derived from its ability to inhibit cytokine-mediated and growth factor-mediated signal transduction in both B and T lymphocytes. Progression through the G1 phase of the cell cycle is blocked, and, as a result, lymphocyte proliferation is inhibited. Unlike CsA and TAC, SRL does not inhibit calcineurin and does not cause nephrotoxicity [15].

SRL has shown potent immunosuppressive activity in a number of in vitro and in vivo models. It demonstrated potent anti-rejection activity and the ability to prolong graft survival in animals [1, 3, 14]. Clinical experience in patients after renal transplantation has been described. The clinical activity of SRL-based therapy in preventing acute rejection, with an acceptable but different safety profile than CsA, was confirmed. Phase-III randomized controlled trials of SRL were conducted that used SRL in combination with full-dose CsA in renal transplantation. This combination was chosen because SRL synergistically increased the inhibitory effect of CsA on lymphocyte proliferation [5]. Both studies showed that the addition of SRL in a fixed-dose regimen of 2 or 5 mg/day halved the rate of acute rejection of renal allografts in the first year [6, 9]. Furthermore, the results suggested that in combination with a short course of MMF, SRL could be used as primary therapy in human renal transplant patients [8].

Experience with SRL after liver transplantation (LTX) is still limited. The combination of SRL with CsA provided potent immunosuppression in liver transplantation [18]. The feasibility of converting stable liver transplant recipients with calcineurin inhibitor toxicity from CsA or TAC to SRL maintenance therapy has been shown [2, 10]. In another study with LTX, recipients were administered SRL in combination with low-dose TAC and steroids, which proved to be an effective combination therapy with very low rates of renal dysfunction and hypertension, and low rates of opportunistic infection [11]. Experience with SRL in combination with MMF after LTX has not yet been described in the literature.

MMF also has mechanisms that target a different stage of the immune response than do CsA and TAC, and offers a further non-nephrotoxic alternative to standard calcineurin-inhibitor-based regimens. In the rat, the combination of MMF and SRL was synergistic in preventing acute heart, pancreas and kidney allograft rejection [17]. The introduction of MMF as a treatment for rejection after liver transplantation has been described [7]. We present here a retrospective follow-up study of late conversion to SRL and MMF as an alternative treatment for liver transplant patients with calcineurin-inhibitor-related side effects.

Methods

From July 2001 to March 2002, seven liver transplant recipients (three female, 59 (41–66) years old) were enrolled in the study. Indications for liver transplantation were hepatitis B and/or hepatitis C (three), alcohol-induced cirrhosis (three) and Wilson's syndrome (hepatolenticular degeneration; one). LTX was performed by standard (four) or piggy-back (three) technique. During the first week after LTX, all patients received horse ATG 1.5 to 3.3 mg/kg per day (lymphoglobulin, Pasteur Merieux) and a methylprednisolone taper starting with 70 mg every 8 h. Calcineurin inhibitors were initiated according to the kidney function on days 1 to 3, and standard trough levels were intended to be reached on day 7. From day 7 on, prednisolone was given at 15 mg/day and tapered after the first month. Steroids were stopped not later than 3 months after LTX, and all patients were off steroids at the time of conversion. MMF was started orally between days 2 and 7, with target MPA trough levels of approximately 1 μg/ml on day 7.

The switch to SRL was performed 62 (37–118) months after LTX. The reasons for the switch from CsA or TAC to SRL were renal (six) and neurological (one) impairment. As immunosuppressive therapy, SRL was administered at trough levels from 4–10 ng/ml, and MMF at trough levels of approximately 1 μg/ml. One patient, who was converted from calcineurin-inhibitor monotherapy, received SRL monotherapy. The mean follow-up time under SRL was 131 (26-251) days. SRL and MMF levels were controlled weekly in the first month and then monthly. The SRL level was measured with the HPLC method using UV-detection, and the MMF level was measured with the HPLC method according to Svensson [16]. Graft function was observed by liver function (total bilirubin, aspartate aminotransferase, alanine aminotransferase, γ glutamyltransferase, alkaline phosphatase) and ultrasound. Rejection was defined as a significant increase of respective laboratory values and would have been confirmed by biopsy. Furthermore, patients were monitored for serum creatinine level, white blood cells, and platelets. Blood pressure and neurological symptoms were also followed. Hypercholesterolaemia was

Table 1 Laboratory values for patients under SRL therapy (*ASAT* aspartate aminotransferase, *ALAT* alanine aminotransferase, *GGT* γ gamma glutamyltransferase, *AP* alkaline phosphatase, *CHE*, cholinesterase, *AT III* antithrombin III)

Patient no.	Time	ASAT (U/l)	ALAT (U/l)	GGT (U/l)	Bilirubin (mg/dl)	AP (U/l)	CHE (U/l)	Quick (%)	AT III (%)
1	Pre-SRL	7	9	37	0.3	190	5,869	112	116
	1 Month post-SRL	7	10	41	0.2	186	6,758	121	121
	3 Months post-SRL	8	10	42	0.2	150	7,248	123	112
2	Pre-SRL	6	6	20	0.6	155	7,720	116	123
	1 Month post-SRL	14	46	153	1.3	346	7,869	114	123
	3 Months post-SRL	12	34	167	1.7	351	7,471	113	123
3	Pre-SRL	14	15	35	1.3	102	5,088	119	106
	1 Month post-SRL	9	6	28	0.7	91	4,864	118	112
	3 Months post-SRL	9	7	17	0.7	82	4,433	116	105
4	Pre-SRL	10	7	99	0.7	137	3,029	92	76
	1 Month post-SRL	12	6	68	0.7	168	3,614	102	71
	3 Months post-SRL	11	6	156	0.5	272	4,148	92	99
5	Pre-SRL	18	34	106	0.6	366	5,589	114	101
	26 Days post-SRL	21	31	103	0.6	476	5,064	123	98
	3 Months post-SRL	–	–	–	–	–	–	–	–
6	Pre-SRL	16	15	29	0.6	122	6,709	117	111
	1 Month post-SRL	19	25	45	0.2	142	8,299	130	121
	3 Months post-SRL	–	–	–	–	–	–	–	–
7	Pre-SRL	11	14	16	0.5	98	7,023	105	123
	1 Month post-SRL	14	19	21	0.5	104	6,777	101	123
	3 Months post-SRL	14	20	25	0.5	107	7,035	108	113

defined as a serum cholesterol level higher than 240 mg/dl, and hypertriglyceridaemia as a serum triglyceride level higher than 300 mg/dl.

Infections were defined by clinical symptoms and as the presence of organisms (bacterial, viral or fungal) that required intravenous therapy. Activity of cytomegalovirus was assessed by the occurrence of pp65-positive cells.

Results

Seven patients were switched from CsA or TAC to SRL after liver transplantation. As immunosuppressive therapy, SRL and MMF were administered to six patients; one received SRL monotherapy. Patient and graft survival during the time of SRL therapy (131 (26–251) days) was 100%; there were no rejection episodes, and no liver biopsies had to be performed. Laboratory values concerning liver function are demonstrated in Table 1.

Renal function improved in five of the six patients (83.3%) with renal impairment. Serum creatinine levels at the initiation of SRL therapy and on days 7, 14, 21, 28, 60, 90, 120, 150, 180, 210, 240 and 270 are shown in Fig. 1. Renal function improved in all patients after initiation of SRL therapy. One patient, who had had a renal transplantation 4 years previously (6 years after LTX), showed the highest creatinine levels before and after the switch to SRL. Another patient had an improvement of creatinine levels in the first weeks after initiation of SRL, and again, there was an impairment. Figure 2 shows the creatinine levels in the patients whom we had to switch back to calcineurin inhibitors (CsA: two, TAC: one) due to side effects. There is an

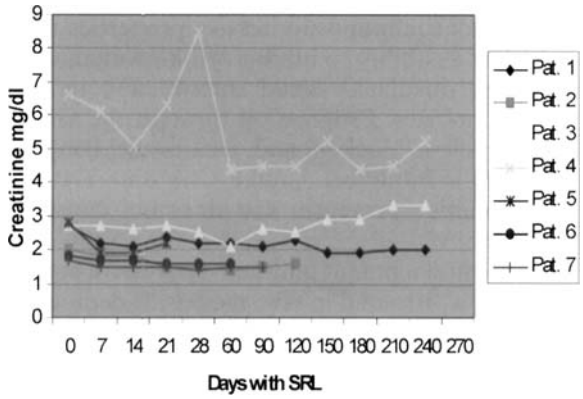

Fig. 1 Creatinine levels under SRL therapy

increase in creatinine after the initiation of calcineurin inhibitors in all patients.

In the patient whom we switched to SRL due to neurological impairment, the neurological function recovered, and renal function improved. During the whole time of observation, no infection became manifest, and no CMV antigenaemia was found. Side effects developed in three patients (42.8%). Hyperlipidaemia was found in two patients, one of them was intolerant of statin lipid-lowering agents and had to be switched back to CsA. The second patient received a lipid-lowering therapy and was continued on SRL therapy. SRL levels at the time of hyperlipidaemia were between 7 and 10 ng/ml. Serum cholesterol and triglyceride levels are shown in Figs. 3 and 4. Severe leucopenia and thrombocytopenia were not found in any patient (Figs. 5 and 6). Cessation of

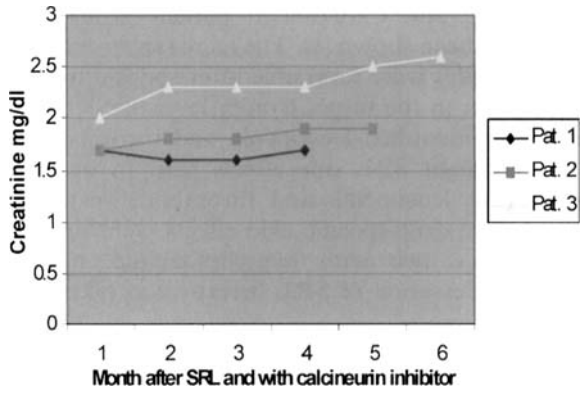

Fig. 2 Creatinine levels with calcineurin inhibitors

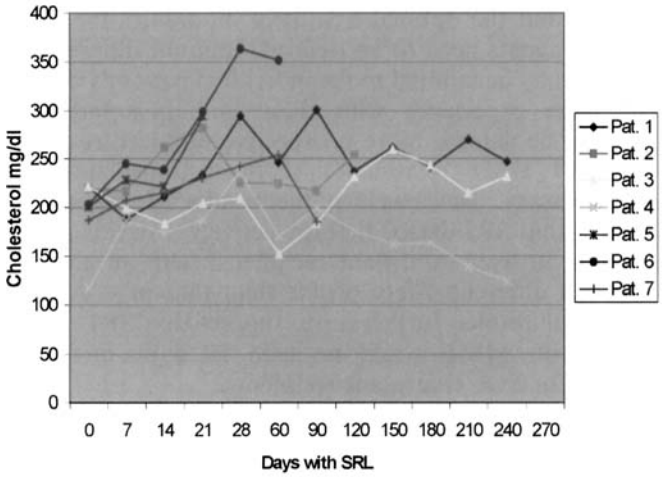

Fig. 3 Cholesterol levels under SRL therapy

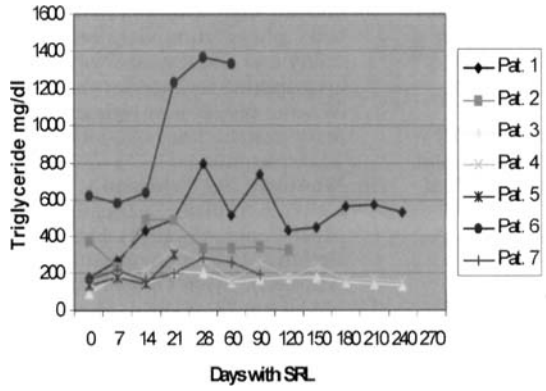

Fig. 4 Triglyceride levels under SRL therapy

therapy became necessary in two patients (exanthema: one, hypertriglyceridaemia: one) because of side effects. SRL levels at the time of exanthema were approximately 13 ng/ml, and at the time of hypertriglyceridaemia, about 8 ng/ml. The patient with SRL monotherapy

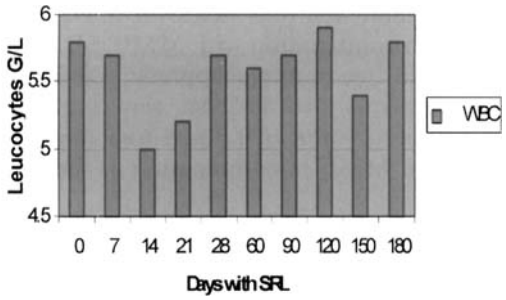

Fig. 5 White blood cells (*WBC*) under SRL therapy

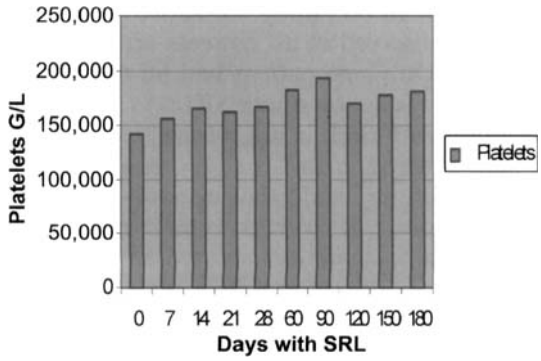

Fig. 6 Platelets under SRL therapy

refused to continue the therapy with SRL. Four patients remained on SRL therapy and are still in the study.

Discussion

In the early years of transplant surgery, graft and patient survival, measured within a 1 to 3-year period after transplantation, were the primary focus. With the introduction of CsA and new surgical techniques, patient and graft survival has improved. With the technical refinements in LTX over the past 20 years, current survival rates exceed 90% for most recipients. The number of liver transplant recipients with long-term survival increases every year. This has broadened the focus for immunosuppression to include such issues as longer graft survival, less chronic rejection and less nephrotoxicity, and has thus improved patient quality of life.

Despite significant improvements in graft and patient survival by means of CsA and TAC, both are nephrotoxic. New non-nephrotoxic agents, such as SRL and MMF, appear promising, but more data are needed to support their use. The data of our study suggest that SRL is a potent immunosuppressive agent of potential benefit in clinical LTX. The safety and efficacy of SRL-based therapy offers an alternative to calcineurin inhibitors and can avoid their specific side effects, such as nephrotoxicity and neurotoxicity. SRL monotherapy

was efficacious and well tolerated over the short period of follow-up. In combination with MMF, SRL provided adequate steroid-free immunosuppression in stable liver transplant recipients. No evidence was found for new toxicities or potentiation of known toxicities from the use of SRL and MMF in combination at these concentrations.

The lack of nephrotoxicity observed in patients treated with SRL suggests that non-nephrotoxic immunosuppressive therapy is an option for patients after liver transplantation. Similarly, studies on MMF-based therapy suggest that this agent may also play a part in non-nephrotoxic immunosuppressive therapy for transplant recipients. In our study, an improvement in renal function was observed in all patients after initiation of SRL therapy. Only one patient had an ongoing deterioration of renal function under SRL. As nephrotoxicity in association with SRL is not known, we suppose that before SRL therapy in this patient, renal function was impaired to such an extent that no long-term improvement was possible. All patients whom we had to switch back to calcineurin inhibitors showed an impairment of renal function. Thus, we conclude that, in contrast to calcineurin inhibitors, SRL offers a non-nephrotoxic immunosuppression. SRL has a different profile of side effects than that for CsA and TAC. Hyperlipidaemia is known to be associated with SRL. Cholesterol and triglyceride levels were higher in SRL patients, but minimal differences in cholesterol and triglyceride levels between SRL and CsA-treated patient groups at 12 months have been shown [8]. The increases in cholesterol and triglycerides were reversible after specific treatment and reduction in the target trough level of SRL. Other studies have identified leucopenia and thrombocytopenia as significant SRL side effects [13]. In our study, no significant leucopenia and thrombocytopenia were observed. The drug-specific side effects were limited to three patients, two with hyperlipidaemia, one with exanthema. Cessation of SRL therapy was necessary in two cases.

The new non-nephrotoxic immunosuppressive drugs effectively prevent acute rejection in liver transplant recipients. Furthermore, they might offer immunosuppressive therapy for patients with renal or neurological impairment after LTX. However, there is much to be learned, and the optimal treatment modalities for each of these agents need to be defined. Immunosuppressive therapy may be tailored to the individual patient's needs. For more experience with these immunosuppressive drugs to be gained, more prospective multicentre trials with SRL/MMF treatment after liver transplantation are necessary. Nevertheless, the results of this study confirm that SRL-based therapy actively prevents acute rejection in liver transplant recipients, with an acceptable, but different safety profile than that of CsA and TAC. Our results, furthermore, suggest that SRL associated with MMF could be used as a maintenance therapy for liver transplant recipients.

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