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RAPID COMMUNICATION

Conversion to sirolimus immunosuppression in liver transplantation recipients with hepatocellular carcinoma: Report of an initial experience

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Abstract

AIM: To report a retrospective analysis of preliminary results of 36 patients who received sirolimus (SRL, Rapamune®, rapamycin) in a consecutive cohort of 248 liver allograft recipients.

METHODS: Thirty-six liver transplant patients with hepatocellular carcinoma (HCC) who were switched to SRL-based immunosuppression therapy from tacrolimus were enrolled in this study. The patients who were diagnosed as advanced HCC before orthotopic liver transplantation (OLT) were divided into group A (n = 11), those who were found to have HCC recurrence and/or metastasis after OLT were assigned to group B (n = 18), and those who developed renal insufficiency caused by calcineurin inhibitor (CNI) were assigned to group C (n = 7) after OLT.

RESULTS: The patients were followed up for a median of 10.4 mo (range, 3.8-19.1 mo) after conversion to SRL therapy and 12.3 mo (range, 5.1-34.4 mo) after OLT. Three patients developed mild acute cellular rejection 2 wk after initiating SRL therapy, which was fully reversed after prednisolone pulse therapy. In group A, only 1 patient was found to have HCC recurrence and metastasis 12 mo after OLT. In group B, 66.7% (12/18) patients (2 with progressive tumor, 7 with stable tumor and 3 without tumor) were still alive due to conversing to SRL and/or resection for HCC recurrence at the end of a median follow-up of 6.8 mo post conversion and 10.7 mo post-transplant. In group C, no HCC recurrence was demonstrated in 7 patients, and renal function became normal after SRL therapy. Thrombocytopenia (n = 2), anemia (n = 8), and oral aphthous ulcers (n = 7) found in our cohort were easily manageable.

CONCLUSION: The conversion to SRL-based immunosuppression may inhibit the recurrence and metastasis of HCC and improve CNI-induced renal insufficiency in OLT patients with HCC.

Key Words: Sirolimus; Orthotopic liver transplantation; Hepatocellular carcinoma

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INTRODUCTION

Sirolimus (SRL, Rapamune®, rapamycin) is a macrocyclic triene antibiotic that was initially found to have antifungal properties, and may act as a primary immune suppressant or antitumor agent. Unlike tacrolimus and cyclosporine, SRL itself does not reduce glomerular filtration rate (GFR) or nephrotoxicity[1]. Liver transplant patients suffering from calcineurin inhibitor (CNI)-related renal insufficiency could be converted to SRL-based monotherapy or combination therapy[2]. SRL has potent antitumor activity *in vitro* and *in vivo*[3-5]. This property may offer a new approach to inhibiting the recurrence and metastasis of hepatocellular carcinoma (HCC) after orthotopic liver transplantation (OLT). Considering these two favorable effects of SRL, we added SRL to our immunosuppressive regimens in November 2003. We here report a retrospective analysis of preliminary results of 36 patients who received SRL in a consecutive cohort of 248 liver allograft recipients. The indications for conversion to SRL-based immunosuppressive regimen with reduction of CNIs included: patients with

advanced HCC before OLT, HCC recurrence or metastasis after OLT, and CNI- induced nephrotoxicity.

MATERIALS AND METHODS

Patients

Between April 2001 and January 2004, a total of 248 patients underwent OLT in our hospital. Among them, 36 (28 men, 8 women) received treatment with SRL. The indication for OLT was HCC combined with hepatitis B-related cirrhosis. These 36 patients were divided into 3 groups: group A: patients with preoperative advanced HCC (n = 11), group B: patients with HCC recurrence and metastasis after OLT (n = 18), group C: patients with CNI-induced renal insufficiency (n = 7). Advanced HCC was defined as stage III-IV according to the pathological TNM classification[6]. Patients were eligible for SRL treatment if the serum creatinine level was ⩾120 μmol/L (normal range, 40-120 μmol/L) on three occasions after exclusion of other causes of nephrotoxicity and after minimization of CNI levels without graft rejection[2]. The study protocol was approved by the Research Ethics Committee of Zhongshan Hospital, and informed consent was obtained from all participants. Patients were followed up for a median of 10.4 mo (range, 3.8-19.1 mo) after conversion to SRL therapy and 12.3 mo (range, 5.1-34.4 mo) after OLT. Chest radiography, abdominal ultrasonography, computed tomographic (CT) scanning and serum alpha-fetoprotein (AFP) measurement were performed once a month during the follow-up period. Liver angiography, chest CT scanning and bone scintigraphy were performed when tumor recurrence or metastasis was suspected.

Immunosuppression

Immunosuppression was started during surgery with 1000 mg methylprednisolone, followed by taper, 240 mg to 40 mg a day for over 6 d. Maintenance prednisone at 5-20 mg daily was then used based on the perceived degree of patient debilitation. Tacrolimus, the routine CNI used in our hospital, was orally administered 12 h after OLT at 0.05-0.1 mg/kg every 12 h. The dose was adjusted to reach target levels of 10-15 ng/mL during the first 14 d. Then the levels were maintained within the range of 6-10 ng/mL during the second 14 d, and 5-8 ng/mL thereafter. Mycophenolate mofetil (MMF) (0.25-0.5 mg, *po*, bid) was used for 12 patients when tacrolimus did not reach the target level after addition of tacrolimus.

Tacrolimus dose was reduced to half, and its concentration was maintained at 2-5 ng/mL. MMF was stopped once SRL therapy was initiated. SRL could impair wound healing in transplant recipients[7]. We started to use SRL in our study at least 1 mo after OLT to avoid affecting the wound healing. All the 36 patients were given SRL at the initial loading dose of 3 mg/m^2. SRL doses were then adjusted to achieve steady-state whole-blood level of approximately 5-8 ng/mL thereafter. SRL oral solution (Wyeth-Ayerst Research, Philadelphia, PA, USA) was administered once daily in the morning, following dilution with water or orange juice. The dose was reduced or increased to 0.5 mg/d if the level was higher than 8 ng/mL or lower than 5 ng/mL.

Table 1 Demographic Data of the patients

	Group A (n = 11)	Group B (n = 18)	Group C (n = 7)
Age (mean±SD yr)	46.6 ± 10.6	47.1 ± 7.8	53.6 ± 5.7
Gender (women/men)	4/7	3/15	1/6
Immunosuppression regimen before switch			
Tacrolimus+steroids+MMF	7	4	1
Tacrolimus+steroids	4	14	6
Immunosuppression regimen after switch			
Sirolimus+tacrolimus	8	13	5
Sirolimus+steroids+tacrolimus	3	5	2

MMF: mycophenolate mofetil.

The patients who were suspected to have rejection underwent liver transplant biopsy. Anti-rejection measures included prednisolone pulse therapy for mild rejection or antibody therapy with thymoglobulin for more severe rejection.

Anti-viral drugs

Lamivudine was started at least 1 wk before OLT (100 mg orally, daily) and continued thereafter. Hepatic B immunoglobulin (800 IU/d) was given intramuscularly for 1 wk, followed by 400 IU twice a week and then once a month according to the anti-HBs titer.

Statistical analysis

Overall survival rate was analyzed using the Kaplan-Meier product limit method. The differences in white blood cell count and serum creatinine levels between the two different groups were evaluated by Mann-Whitney U test. All data were analyzed by SPSS 10.0 (SPSS Inc, Chicago, IL), and $P < 0.05$ was considered statistically significant.

RESULTS

Patient demographics are summarized in Table 1. Tumor extension and histopathological evaluation are summarized in Table 2.

In group A, 11 advanced HCC patients (6 with tumor thrombus in the first right branch of portal vein) were switched to SRL in the early stage (range, 1.0-6.0 mo) after OLT. Only 1 patient was found to have tumor recurrence in liver graft and metastasis in bone and lung 12 mo after OLT, and died at 14 mo posttransplant. The other 10 patients survived without tumor during a follow-up period of 3.9-11.5 mo after conversion to SRL therapy and 5.1- 3.5 mo after OLT.

In group B, 18 patients had tumor recurrence and metastasis within 8 mo after OLT, and were conversed to SRL-based immunosuppression. The overall 1-year survival rate was 68.2%, and 66.7% (12/18) patients were still alive after a median follow-up of 6.8 mo (range, 3.8-14.2 mo) post conversion and 10.7 mo (range, 5.6-20.1 mo)

Table 2 Tumor status at the time of transplantation

	Group A (n = 11)	Group B (n = 18)	Group C (n = 7)
Stage			
I	0	0	4
II	0	0	3
III	4	9	0
IV	7	9	0
Grade (WHO)			
I	1	0	4
II	6	10	3
III	4	8	0
PVTT			
Yes	6	6	0
No	5	12	7

Stage according to pTNM classification[6]; Grade according to WHO definition (grade I: well-differentiated; grade II: moderately-differentiated; grade III, poorly-differentiated); PVTT: portal vein tumor thrombus.

posttransplant. Four patients who had a single nodule of lung metastasis after transplantation underwent local lung resection. After resection, 2 patients were found to have multiple metastases in the lung again and received bronchial arteriographic embolization, and survived for 5 mo and 9 mo with tumor progression. The other 2 patients remained tumor free at 3 mo and 10 mo after resection for lung metastasis. Another patient who was found to have bone (transverse process of the twelfth thoracic vertebra) metastasis 1 mo after OLT underwent metastasis resection and local radiotherapy. She survived without tumor for 11 mo after conversion and 12 mo after OLT. The other 13 patients had multiple organ (liver and lung) recurrence and metastasis posttransplant, and could not undergo surgical ablation. While no additional therapy beyond SRL was given, 53.8% patients (7/13) were still alive, their tumors remained stable in size after SRL, and 46.2% patients (6 /13) died due to the progression of tumor.

In group C, the conversing SRL time for patients with CNI-induced renal insufficiency CNI was different (range, 1.5-17 mo). No tumor recurrence was demonstrated in the 7 cases. Improvement in renal function was found in 7 patients with their serum creatinine levels returned to normal within 60 d after SRL (154.0 ± 36.2 μmol/L *vs* 83.6 ± 11.4 μmol/L, $P < 0.05$). The most significant improvement was achieved during the first 30 d.

In all the 36 patients, only 3 patients developed biopsy-proven mild acute cellular rejection (Banff criteria rejection activity index of 3-4) 2 wk after initiating SRL therapy, which was fully reversed with prednisolone pulse therapy. All the patients were HBsAg negative and HBsAb positive after OLT after receiving lamivudine and hepatic B immunoglobulin treatment. There was no significant decrease in white blood cell count (6137 ± 1974 c/μL *vs* 5725 ± 1642 c/μL, $P > 0.05$). Eight patients developed anemia (hemoglobin < 10 g/L), and 5 received with red blood cell transfusion, 3 had percutaneous injection of recombinant erythropoietin. Platelet count decreased significantly as compared with baseline in 2 patients (from 125.1×10^9/L to 43.3×10^9/L and from 103.6×10^9/L to 51.2×10^9/L, respectively). The other side effect observed was oral aphthous ulcer (7/36). The symptoms of oral aphthous ulcer improved with a dose reduction of SRL in 2 patients. Relief therapies included antiviral and topical anesthetics in monotherapy or combined therapy in 5 patients. No incidence of hepatic artery thrombosis, delay in wound healing or other allograft dysfunction was found. No other serious hematologic or biochemical changes were observed in this study.

DISCUSSION

The use of SRL in chemotherapy or prevention of tumor proliferation is intriguing[8, 9]. The immunosuppressive and antitumor effects of SRL share a common mechanism of action. SRL inhibits the mammalian target of SRL (mTOR), which prevents acute graft rejection mediated by interleukin-2 and blocks other cytokine signal transduction, thus directly inhibiting tumor cell proliferation[9]. The effects are supported by clinical results with SRL in organ transplantation. Kneteman *et al*[5] followed up 21 patients with HCC beyond Milan criteria who underwent OLT and were treated with a SRL-based immunosuppressive protocol. The 1- and 4-year survival rate was 90.5% and 82.9%, respectively. Only 4 patients were found to have tumor recurrences at a median time of 17 mo postplantation, and the median postrecurrence survival time was 15.5 mo. Compared with the survival rate reported by Yao *et al*[10], our initial experience also suggests that SRL therapy has beneficial effects on HCC patient survival. In group A, only 1 patient died at month 14 posttransplant. The other 10 patients acquired tumor-free survival during a follow-up period of 3.9-11.5 mo after conversion and 5.1-13.5 mo after OLT. In group B, the 1-year survival rate was 68.2% and 66.7% (12/18) patients were still alive (2 with progressive tumor, 7 with stable tumor, and 3 without tumor) after SRL therapy (5 patients had resection for HCC recurrence) after a median follow-up of 6.8 mo (range, 3.8-14.2 mo) post conversion and 10.7 mo (range, 5.6-20.1 mo) posttransplant.

Suppression of the immune system by antirejection therapy has long been linked to increased rates of cancer in transplant recipients. Advances in basic medical sciences have strengthened the association with CNI and increased risk of malignant disease recurrence[11, 12]. Hojo *et al*[12] reported that cyclosporine transforms a noninvasive lung cancer cell line into an invasive phenotype. Vivarelli *et al*[13] reported that patients with HCC receiving a high dosage of cyclosporine during post-liver transplant 3 mo to 12 mo experience a significantly lower recurrence-free survival. These results indicate that cyclosporine can exacerbate tumor growth in patients with malignant tumor[14]. Schumacher *et al*[15] recently reported that *in vitro* suppression of human hepatoma cells by SRL, in contrast to tacrolimus, inhibits hepatoma growth. Our immunosuppressive protocol was planned to minimize the potential impact of steroids and CNIs on HCC recurrence, and to allow for benefits of the previously-reported antitumor

activity of SRL[16]. In group A, only 1 patient who had a tumor (14 cm in diameter) with tumor thrombus in the main branch of portal vein in the explant liver, was found to have recurrence and metastasis 12 mo after OLT. The other 10 advanced HCC patients including 5 with tumor thrombus in the main branch of portal vein have survived without tumor recurrence and metastasis till now (range, 5.1-13.5 mo after OLT). While in group B, 18 advanced HCC patients were found to have intrahepatic recurrence or extrahepatic metastases (mainly in lung) within 8 mo after OLT on maintenance immunosuppression with tacrolimus and steroids. We have also made a retrospective analysis of 40 patients with advanced HCC, including pTNM stage III in 21 patients, and pTNM stage IV in 19 patients, and 9 patients with tumor thrombus in the main branch of portal vein, who underwent OLT before adding SRL to our immunosuppressive regimens. HCC recurrence and metastasis were observed in 11 patients after OLT (11/40, recurrence rate 27.5%), which occurred within 8 mo[17]. As such, recurrence time seemed to be later (≤8 mo *vs* 12 mo) in patients with SRL-based immunosuppression. The difference may be most probably ascribed to the antitumor effect of SRL. However, our study is limited in terms of the number of patients and time of follow-up to properly define the effect of antitumor. Nonetheless, SRL offers a new and promising approach to the prevention or management of posttransplant recurrence and metastasis of HCC. The immunosuppressive efficacy and antitumor activity of SRL may confer a unique for the long-term management of OLT for HCC.

In many open-label studies on liver transplant recipients using SRL as part of a primary immunosuppressive regimen, the occurrence of acute cellular rejection is relatively low. Watson *et al*[18] reported the none of their patients on triple-therapy experiences rejection. Similarly, McAlister *et al*[19] reported that rejection is observed in only 14% patients, which is approximately 50% lower than that in historic patients with conventional immunosuppression. During the median follow-up period of 10.4 mo after conversion to SRL therapy in our cohort, only 3 of 36 patients (8.3%) presented with acute rejection episode, no patient had steroid resistant rejection, and no grafts were lost. These results suggest that SRL-based immunosuppressive protocol is effective in preventing acute cellular rejection.

Renal dysfunction after OLT continues to plague the improvements in patients and graft survival among transplant recipients, and occurs in any different period after OLT. The incidence of chronic renal insufficiency in liver transplant recipients is 0.8% per year, ultimately leading to hemodialysis in 10% of cases and a mortality of 44%[20]. The nephrotoxicity following OLT is likely due to many factors, including hypotension during operation, infection, concomitant use of other nephrotoxic agents. However, the use of CNI such as tacrolimus is a major cause[21]. SRL appears to be devoid of nephrotoxic effects, while demonstrating comparable immunosuppression with CNI[22]. In group C, only after exclusion of other causes of nephrotoxicity and minimization of tacrolimus levels without graft rejection, could the patients be converted to SRL-based immunosuppression, suggesting that the improvement of renal function can not be derived simply from the tacrolimus dose reduction without graft rejection. Our results showed that serum creatinine level in nephrotoxicity group improved significantly after SRL therapy. All the 7 patients had complete normalization within 60 d after SRL treatment, suggesting that the observed improvement of renal function derives from both SRL conversion and tacrolimus dose reduction without graft rejection. Besides, in groups A and B, 29 patients who did not develop renal insufficiency during the SRL therapy further confirmed the non-nephrotoxicity of SRL.

The side-effect profile of SRL differs from that of CNIs. In our study, thrombocytopenia was easily reversible. Anemia could be controlled with blood cell transfusions, and recombinant erythropoietin. Oral aphthous ulcers were treated successfully with a reduced SRL dose and relief therapies. These adverse effects did not require discontinuation of SRL therapy and could be manageable. Hepatic artery thrombosis (HAT) is a dreaded complication, because it is often associated with allograft loss, patient death, or both. However, a single-center report with SRL and tacrolimus in University of Edmonton showed a low incidence of HAT (2%)[18]. In University of Colorado, Dunkelberg *et al*[23] noted that 170 patients with SRL treatment (5.3%) develop HAT compared with 8.3% of historical control patients. In both the Edmonton and the Colorado experience, the dose of SRL is modest (maintenance of 2 mg/d) and SRL levels are generally low (7 ng/mL). Considering that the adverse effects related to the administration of SRL are dependent on dose and concentration, we established the low target level of SRL in our group (5-8 ng/mL). With this approach, no episode of HAT was seen in our study. However, further investigation is necessary for elucidating the relationship between HAT and SRL.

In conclusion, SRL-based immunosuppression may improve CNI-induced renal insufficiency, inhibit the recurrence and metastasis of HCC, and prolong the patients' survival with good tolerability. However, further prospective studies with a large number of HCC patients with long-term SRL therapy are needed to confirm these findings.

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