Comparison of Early and Late Conversion of Sirolimus in Experimental Model of Chronic Cyclosporine Nephropathy

Jin Young Kim¹*, Jung Yeon Ghee¹*, Sun Woo Lim¹, Shang Guo Piao¹, Byung Ha Chung¹, Hye Eun Yoon¹, Hyeon Seok Hwang¹, Bum Soon Choi¹, Jin Kim¹, and Chul Woo Yang¹

¹Transplant Research Center, Convergent Research Consortium for Immunologic Disease; ²Cell Death Disease Research Center, Department of Anatomy, The Catholic University of Korea, Seoul, Korea

*Jin Young Kim and Jung Yeon Ghee contributed equally to this work.

Received: 1 June 2011
Accepted: 21 November 2011

Address for Correspondence:
Chul Woo Yang, MD
Department of Internal Medicine, Seoul St. Mary’s Hospital, The Catholic University of Korea, 222 Banpo-dong, Seocho-gu, Seoul 137-040, Korea
Tel: +82.2-530-2527, Fax: +82.2-536-0323
E-mail: yangch@catholic.ac.kr

This study was supported by a grant of the Korea Healthcare Technology R&D project, Ministry for Health & Welfare Affairs, Republic of Korea (A092258).

INTRODUCTION

Sirolimus (SRL) is a promising maintenance immunosuppressive agent for replacing calcineurin inhibitors (CNI) (1-4). SRL itself does not cause severe nephrotoxicity, but combined SRL and CNI treatment causes significant nephrotoxicity in most animal and human studies (4-8). There is an emerging consensus that conversion from a CNI to SRL is an effective strategy for reducing CNI-induced nephrotoxicity.

Clinical trials of SRL show that early conversion to SRL is effective in preserving graft function and that late conversion in recipients with poor graft function or proteinuria does not provide beneficial effects on graft function (9, 10). Therefore, early conversion to SRL after transplantation is recommended, but the optimal time for conversion has not been determined. It is also unclear whether the undesirable effects of late conversion to SRL are related to the preexisting CNI-induced renal injury or to the effect of SRL itself.

In this study, we focused on the influence of SRL on Cyclosporine (CsA)-induced renal injury. To evaluate the optimal time for conversion, we compared the influence of early and late conversion from CsA to SRL on CsA-induced renal injury in an experimental model of chronic CsA-nephrotoxicity. The results of our study show clearly that early conversion from CsA to SRL is effective in preventing CsA-induced renal injury in an experimental setting of CsA-induced renal injury.
Ltd, Basel, Switzerland) was diluted in olive oil (Sigma Co., St Louis, MO, USA) to a final concentration of 15 mg/mL. SRL (Wyeth-Ayerst Research, Princeton, NJ, USA) was dissolved in solution of Tween 80 (10%), N, N-dimethylacetamide (20%), and polyethylene glycol 400 (70%), to a final concentration of 0.3 mg/mL.

**Experimental design**

Three separate experiments were performed, as shown Fig. 1.

*Experiment I:* This experiment was designed to evaluate the influence of the combined treatment of SRL and CsA on CsA-induced nephrotoxicity. Rats were randomized to four groups and treated for 4 weeks:

1) Vehicle group (VH4, n = 7): rats received a daily subcutaneous injection of olive oil (1 mL/kg) for 4 weeks.
2) VH4 + SRL4 group (n = 7): rats received a daily subcutaneous injection of olive oil and SRL (0.3 mg/kg) for 4 weeks.
3) CsA4 group (n = 7): rats received a daily subcutaneous injection of CsA (15 mg/kg) for 4 weeks.
4) CsA4 + SRL4 group (n = 7): rats received a daily subcutaneous injection of CsA and SRL (0.3 mg/kg) for 4 weeks.

*Experiment II:* This experiment was designed to evaluate the effect of early conversion from CsA to SRL on CsA-induced nephrotoxicity. Rats were randomized to four groups and treated for 4 weeks:

1) VH1 + W3 group (n = 7): rats received olive oil for 1 week, after which olive oil was withheld for 3 weeks.
2) VH1 + SRL3 group (n = 7): rats received VH for 1 week and then SRL for 3 weeks.
3) CsA1 + W3 group (n = 7): rats received CsA for 1 week, after which CsA was withheld for 3 weeks.
4) CsA1 + SRL3 group (n = 7): rats received CsA for 1 week and then SRL for 3 weeks.

*Experiment III:* This experiment was designed to evaluate the effect of late conversion from CsA to SRL in established chronic CsA nephropathy. Rats were randomized to four groups and treated for 4 or 8 weeks:

1) VH4 + W4 group (n = 7): rats received a daily subcutaneous injection of olive oil for 4 weeks, after which olive oil was withheld for 4 weeks.
2) VH4 + SRL4 group (n = 7): rats received a daily subcutaneous injection of olive oil for 4 weeks, and then SRL for 4 weeks.
3) CsA4 + W4 group (n = 7): rats received a daily subcutaneous injection of CsA for 4 weeks, after which the CsA was withheld for 4 weeks.
4) CsA4 + SRL4 group (n = 7): rats received a daily subcutaneous injection of CsA for 4 weeks, and then SRL for 4 weeks.

The doses and duration of administration of the vehicle (Olive

![Fig. 1. Experimental design. Three separate studies were performed. (A) Combined sirolimus and cyclosporine, (B) early conversion, and (C) late conversion. S.C., subcutaneous injection; VH, vehicle.](http://dx.doi.org/10.3346/jkms.2012.27.2.160)
oil; 1 mL/kg). CsA (15 mg/kg), and SRL (0.3 mg/kg) were chosen based on previous reports (5, 11, 12).

Measurement of renal function and whole blood CsA and SRL levels
Serum creatinine (SCr) concentration was measured using a Cobas autoanalyzer (Roche Diagnostics, Division of Hoffmann-La Roche Inc., Nutley, NJ, USA). Whole-blood CsA level was measured using a monoclonal radioimmunoassay (INSCTART Corp., Stillwater, MN, USA), and whole-blood SRL concentration was measured using a microparticle enzyme immunoassay (Abbott Diagnostics, Abbott Park, IL, USA).

Preservation of kidney
The kidneys were preserved by in vivo perfusion through the abdominal aorta. In brief, the animals were perfused with 0.01 M phosphate-buffered saline (PBS) to wash out the blood. The left kidney was removed for immunoblotting analysis or RNA extraction and the right kidney was removed after perfusion with the periodate-lysine-parafomaldehyde (PLP) solution for 4 min. The kidneys were removed and cut into sagittal slices of 1-2 mm thickness and post-fixed overnight in PLP solution at 4°C. A part of the PLP-fixed tissues was embedded in wax for trichrome staining. After dewaxing, 4-μm sections were processed and stained with Masson’s trichrome stain.

Measurement of interstitial fibrosis
To assess interstitial fibrosis, kidney paraffin sections were stained with Masson’s trichrome stain. Tubulointerstitial fibrosis (TIF) was identified using the definition described previously (13).

Immunohistochemistry of osteopontin (OPN) and ED-1
After dewaxing, sections were incubated with 0.5% Triton X 100/PBS solution and washed with PBS, and then incubated for 2 hr at 4°C in mouse antiserum against OPN (MPIIB10, obtained from the Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA) and ED-1 (Serotec Inc., Oxford, UK). The number of ED-1-positive cells was quantified per 0.5 mm² area of rat kidney using a computer program (TDI Scope Eye Version 3.0 for Windows, Olympus). A minimum of 20 fields per section were assessed.

Immunoblotting of active caspase-3
Western blot analysis was performed as described previously (13). Active caspase-3 was detected by incubating for 1 hr with a specific antibody (Chemicon International, Inc., MA, USA). Antibody-reactive protein was detected using enhanced chemiluminescence (ECL, Amersham Biosciences, Little Chalfont, UK). Optical density was measured using the VH group as 100% reference and normalized by β-actin.

Northern blot analysis of OPN
Northern blotting was performed as described previously by our laboratory (11). The densitometric analysis was performed using the NIH ImagePC program for three determinations for each band, and the results were corrected to 18S.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining
Cells undergoing apoptosis were identified by the ApopTag in situ apoptosis detection kit (Oncor Inc., Gaithersburg, MD, USA). The number of TUNEL-positive cells was counted in 20 different fields in each section under × 200 magnification (13, 14).

Statistical analysis
The data are expressed as mean ± SEM. Multiple comparisons between groups were performed using one-way analysis of variance followed by Bonferroni post hoc testing (SPSS software version 9.0). Significance was assumed as P < 0.05.

RESULTS
Effect of combined treatment with SRL and CsA on chronic CsA nephropathy
Table 1 shows the basic parameters for the first experimental group. After 4 weeks, the CsA group showed deterioration of renal function, as shown by an increase in SCr concentration com-

Table 1. Effect of combined CsA and SRL treatment on basic parameters

| Parameters          | VH4   | VH4 + SRL4 | CsA4   | CsA4 + SRL4 |
|---------------------|-------|------------|--------|-------------|
| BW (g)              | 327 ± 6 | 284 ± 4 | 314 ± 7 | 228 ± 4   |
| SCr conc (mg/dL)    | 0.51 ± 0.09 | 0.44 ± 0.13 | 0.98 ± 0.03* | 1.21 ± 0.04*† |
| Water intake (mL/day)| 16 ± 2 | 27 ± 3* | 14 ± 2 | 59 ± 6i    |
| Urine volume (mL/day)| 14 ± 4 | 28 ± 4* | 15 ± 2 | 60 ± 7i    |
| CsA conc (ng/mL)    | -     | -       | 2047 ± 63 | 1989 ± 69  |
| SRL conc (ng/mL)    | -     | 12.2 ± 1.1 | -     | 14.2 ± 1.6 |

Values are means ± SE. *P < 0.01 vs VH; †P < 0.05 vs CsA group. BW, body weight; SCr conc, serum creatinine concentration; CsA conc, cyclosporine concentration. SRL conc, sirolimus concentration.
pared with the VH4 and VH4 + SRL4 groups (*P < 0.05). As expected, the combined CsA and SRL treatment significantly impaired renal function compared with the other groups (*P < 0.05).

Kidney tissues from CsA-treated rats had typical striped interstitial fibrosis. Tissues from rats treated with combined CsA4 and SRL4 showed more interstitial fibrosis (39 ± 4/0.5 mm²) com-

![Figure 2](http://dx.doi.org/10.3346/jkms.2012.27.2.160)  
**Fig. 2.** Influence of combined treatment of SRL and CsA on interstitial fibrosis in chronic CsA nephropathy. (A) Trichrome staining. The CsA group shows typical striped interstitial fibrosis in the cortex whereas the VH4 and VH4 + SRL4 groups do not show any change. The CsA4 + SRL4 group shows further interstitial fibrosis compared with the CsA4 and VH4 + SRL4 groups (original magnification, × 200). (B) Quantitative analysis of TIF. Note the markedly greater interstitial fibrosis in the CsA4 + SRL4 group compared with the CsA4 and VH4 + SRL4 groups. *P < 0.01 vs VH4 or VH4 + SRL4 groups; †P < 0.01 vs CsA4 group.

![Figure 3](http://dx.doi.org/10.3346/jkms.2012.27.2.160)  
**Fig. 3.** Influence of combined SRL and CsA treatment on interstitial inflammation in chronic CsA nephropathy. (A) ED-1 immunohistochemistry. (B) Quantitative analysis of ED-1 positive cells. Note the significantly higher number of ED-1 positive cells in the CsA4 + SRL4 group than the CsA4 group. *P < 0.01 vs VH4 or VH4 + SRL4 groups; †P < 0.05 vs CsA4 group. (C) Immunohistochemistry of OPN protein. (D) Northern blot analysis for osteopontin (OPN) mRNA. Note the greater OPN mRNA expression in the CsA4 + SRL4 group compared with the CsA4 and VH4 + SRL4 groups. The data are presented as relative optical density with the VH4 group designated as 100% reference and are normalized to 18S. *P < 0.01 vs VH4 or VH4 + SRL4 groups; †P < 0.05 vs CsA4 group.
pared with tissues from rats treated with SRL alone (0.1 ± 0.04/0.5 mm²) and CsA alone (24 ± 0.8/0.5 mm²) (P < 0.01, Fig. 2). We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used immunohistochemistry to stain for ED-1 to detect macrophage infiltration in this chronic CsA nephropathy model (13, 14). As shown in Fig. 3A, ED-1-positive cells were observed rarely in the VH4 and VH4 + SRL4 groups. More ED-1-positive cells (68 ± 7/mm²) were observed in kidneys from the CsA4 group than from the VH4 group (14 ± 2/mm²), and VH4 + SRL4 group (15 ± 1/mm²) (P < 0.01). The highest number of ED-1-positive cells was observed in the CsA4 + SRL4 group compared with CsA4 group (89 ± 5/mm², P < 0.05).

We used Northern blot analysis to measure the mRNA expression for OPN, a proinflammatory cytokine in animal models of CsA-induced renal injury (15, 16). OPN mRNA was expressed minimally in the kidneys of the VH4 and VH4 + SRL4 groups. The expression of OPN mRNA was significantly higher in the CsA4 group (1,987% ± 179%) than in the VH4 group (100% ± 0.4%) and VH4 + SRL4 group (103% ± 3%) (P < 0.01 for the CsA4 group compared with other two groups). OPN mRNA expression was much higher in the CsA4 + SRL4 group (3,315% ± 361%) than in the VH4, VH4 + SRL4 (P < 0.01), and CsA4 groups (P < 0.05, Fig. 3D). OPN protein expression followed a similar pattern (Fig. 3C).

We used TUNEL staining and active caspase-3 expression for evaluation of apoptotic cell death, one of the mechanisms involved in the injury and repair process in the chronic CsA nephrotoxicity model (17). Treatment with VH or SRL did not affect TUNEL-positive cells, which were observed rarely in the VH4 and VH4 + SRL4 groups (11 ± 0.7/0.5 mm² and 12 ± 0.5/0.5 mm², respectively). However, the number of TUNEL-positive cells was higher in the CsA4 group (54 ± 2/0.5 mm², P < 0.01 vs VH4 or VH4 + SRL4 groups), and even higher in the CsA4 + SRL4 group (84 ± 2/0.5 mm², P < 0.01 vs VH4 or VH4 + SRL4; P < 0.05 vs CsA4, Fig. 4A, B). Immunoblotting analysis of the kidney showed a significant increase in active caspase-3 in the CsA4 group (3,541% ± 473%) compared with the VH4 (103% ± 11%) and VH4 + SRL4 group (105% ± 9%) (P < 0.01 for the CsA4 group compared with other two groups).
the other two groups). The expression of active caspase-3 was upregulated more in the CsA4 + SRL4 group (5,625% ± 473%) than in the CsA4 group (P < 0.05, Fig. 4C).

**Effect of early conversion on CsA-induced renal injury**

Table 2 shows the effect of early conversion from CsA to SRL on CsA-induced renal injury. Renal function did not differ between CsA early withdrawal (CsA1 + W3 group) and early conversion (CsA1 + SRL3 groups). As shown in Fig. 5, the number of ED-1 positive cells, OPN mRNA and TIF score did not differ between CsA1 + W3 and CsA1 + SRL3 groups. Fig. 6 shows the number of TUNEL-positive cells and active caspase-3 expression in each experimental group. As expected, the number of TUNEL-positive cells and active caspase-3 expression did not differ significantly between CsA1 + W3 and CsA1 + SRL3 groups.

**Effect of late conversion on progression of chronic CsA nephropathy**

Table 3 shows the effect of late conversion from CsA to SRL on the progression of chronic CsA nephropathy. Renal function was normalized after CsA withdrawal (CsA4 + W4 group), but late conversion of SRL (CsA4 + SRL4 group) caused deterioration of renal function as compared with the other groups (P < 0.05).

Fig. 7 shows the number of ED-1-positive cells in the experimental groups. The number of ED-1-positive cells was significantly higher in the CsA4 + W4 group (10 ± 1/0.5 mm²) than the VH4 + W4 group (0.6 ± 0.1/0.5 mm²) and VH4 + SRL4 group (0.6 ± 0.1/0.5 mm²) (P < 0.01 for CsA4 + W4 compared with other two groups). Switching CsA to SRL after 4 weeks increased the number of ED-1-positive cells to 32 ± 5/0.5 mm² (P < 0.01). Northern blot analysis showed a significantly greater OPN mRNA expression in the CsA4 + W4 group (151% ± 17%) compared with the VH4 + W4 (128% ± 28%) and VH4 + SRL4 groups (89% ± 35%) (P < 0.01 for the CsA4 + W4 compared with the two other groups). OPN mRNA expression was the highest in the late conversion from CsA to SRL (CsA4 + SRL4) group (691% ± 510%, P < 0.01).
Kim JY, et al. • Effect of Sirolimus on CsA-induced Nephrotoxicity

compared with the CsA4 + W4 group, Fig. 7B).

The TIF score was higher in the CsA4 + W4 group (7.6 ± 1.1/0.5 mm²) compared with the VH4 + W4 (1.8 ± 0.3/0.5 mm²) or VH4 + SRL4 groups (1.7 ± 0.5/0.5 mm²; *P < 0.01 for the CsA4 + W4 group vs. VH4 + W4 or VH4 + SRL4 groups). The TIF score was higher in the CsA4 + W4 group (7.6 ± 1.1/0.5 mm²) compared with the VH4 + W4 (1.8 ± 0.3/0.5 mm²) or VH4 + SRL4 groups (1.7 ± 0.5/0.5 mm²; *P < 0.01 for the CsA4 + W4 group vs. VH4 + W4 or VH4 + SRL4 groups).

![Diagram](http://jkms.org)

**Fig. 6.** Effect of early conversion from CsA to SRL on apoptotic cell death. (A) Quantitative analysis of TUNEL-positive cells. The number of TUNEL-positive cells is slightly higher in the CsA early withdrawal group than in the VH group (*P < 0.01 vs VH1 + W3 or VH1 + SRL3 group). The number of TUNEL-positive cell does not differ significantly between the CsA early withdrawal and early conversion groups. (B) Immunoblot analysis for active caspase-3. Active caspase-3 protein levels do not differ between the CsA early withdrawal and early conversion groups. Active caspase-3 protein levels were referenced against β-actin and the relative optical density is presented with the VH1 + W3 group designated as 100% reference and normalized with β-actin.

![Diagram](http://jkms.org)

**Fig. 7.** Effect of late conversion from CsA to SRL on interstitial inflammation and fibrosis. (A) Quantitative analysis of ED-1-positive cells. The number of ED-1-positive cells is high in the CsA4 + W4 group compared with the VH4 + W4 group or the VH4 + SRL4 group. Late conversion from CsA to SRL at 4 weeks further increases the number of ED-1-positive cells compared with the CsA late withdrawal group. (B) Northern blotting for OPN mRNA. Late conversion from CsA to SRL significantly increases OPN mRNA expression in the CsA late withdrawal rat kidney. The data are presented as relative optical density with the VH4 + W4 group designated as 100% reference and are normalized to 18S. *P < 0.01 vs VH4 + W4 or VH4 + SRL4 group; †P < 0.05 vs CsA4 + W4 group. (C) Quantitative analysis of TIF. Late conversion from CsA to SRL causes greater interstitial fibrosis than that observed in the CsA late withdrawal group. *P < 0.01 vs VH4 + W4 or VH4 + SRL4 groups; †P < 0.05 vs CsA4 + W4 group.
W4 group compared with the two other groups). The TIF score was highest in the late conversion from CsA to SRL group (16 ± 1/0.5 mm²) than in the CsA + W4 group (7.6 ± 1.1/0.5 mm², P < 0.01, Fig. 7C).

Fig. 8A shows the number of TUNEL-positive cells in each experimental group. The number of TUNEL-positive cells was significantly higher in the CsA4 + W4 group (24 ± 2/0.5 mm²) compared with the VH4 + W4 group (4 ± 0.6/0.5 mm²) and VH4 + SRL4 group (4 ± 0.9/0.5 mm², P < 0.01 for the CsA4 + W4 group compared with the two other groups). The number of apoptotic cells was even higher in the late conversion from CsA to SRL group (89 ± 3/0.5 mm², P < 0.05 compared with the CsA4 + W4 group). Active caspase-3 protein levels were significantly higher in the CsA4 + W4 group (407% ± 70%) than the VH4 + W4 group (105% ± 5%) and the VH4 + SRL4 groups (115% ± 10%) (P < 0.01 for the CsA4 + W4 group compared with the two other groups). Active caspase-3 protein level was higher in the CsA4 + SRL4 group (1,029% ± 71%) compared with the CsA4 + W4 group (407% ± 70%, P < 0.05, Fig. 8B).

**DISCUSSION**

Our study was performed to determine optimal timing of conversion from CsA to SRL in experimental model of chronic CsA nephropathy. The results of our study demonstrate clearly that early conversion to SRL attenuates the progression of CsA-induced renal injury, whereas late conversion to SRL does not provide beneficial effects. This finding suggests that the conversion from SRL to CsA should occur as early as possible, before the development of chronic renal injury by CsA.

SRL by itself does not cause serious renal injury, but combined SRL and CsA treatment has a synergic effect on the development of chronic CsA nephrotoxicity (4-8). We first tested whether our experimental model of chronic CsA nephropathy produces similar results to those observed in clinical studies. SRL treatment alone did not induce significant nephrotoxicity but co-administration of CsA and SRL exacerbated CsA-induced renal injury. These results are consistent with the results of clinical studies, indicating that our model is suitable for studying SRL conversion.

To determine the optimal timing for conversion from CsA to SRL, we compared the effect of early and late conversion to SRL on CsA-induced renal injury. We chose day 7 as the time for early conversion and day 28 as the time for late conversion because CsA treatment for 4 weeks in our model induces renal dysfunction and chronic changes in kidney, whereas CsA treatment for 1 week causes minimal changes in renal function and histology (18, 19). In our current study, early conversion from CsA to SRL did not cause further significant changes in renal functional or histology alterations compared with the early CsA withdrawal group. By contrast, late conversion from CsA to SRL did not improve renal function and histopathology (9, 10). Actually, late conversion to SRL aggravated CsA-induced renal injury, as shown by a significant increase in interstitial inflammation and fibrosis, even after CsA withdrawal. This finding implies that the severity of CsA-induced renal injury is an important factor in the successful conversion to SRL.

A CONVERT study is to evaluate the effect of conversion from CsA to SRL at different time points after transplantation (6-120 months) (9), and the effect of SRL conversion was evaluated in terms of graft function or degree of proteinuria. Our experimen-
The experimental model of chronic CsA nephropathy was assessed by comparing CsA and SRL groups. Early conversion to SRL, however, changed macrophage infiltration and OPN expression compared to CsA withdrawal. Late conversion potentiated macrophage infiltration and OPN expression. In conclusion, early conversion to SRL protects against CsA nephrotoxicity.

**REFERENCES**

1. Marcén R, Pascual J, Teruel JL, Villafruela JJ, Rivera ME, Mampaso E, Burgos FI, Ortúñio J. Outcome of cadaveric renal transplant patients treated for 10 years with cyclosporine: is chronic allograft nephropathy the major cause of late graft loss? Transplantation 2001; 72: 57-62.
2. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. N Engl J Med 2002; 346: 580-90.
3. Kreis H, Oberbauer R, Campistol JM, Mathew T, Daloze P, Schena FP, Burke JT, Brault Y, Gioud-Paquet M, Scarola JA, Neylan JF; Rapamune Maintenance Regimen Trial. Long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal. J Am Soc Nephrol 2004; 15: 809-17.
4. Mota A, Arias M, Taskinen EI, Paavonen T, Brault Y, Legendre C, Claeson K, Castagneto M, Campistol JM, Hutchison B, Burke JT, Yiğmez S, Hiýrry P, Neylan JF; Rapamune Maintenance Regimen Trial. Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years. Am J Transplant 2004; 4: 953-61.
5. Shihab FS, Bennett WM, Yi H, Choi SO, Andoh TF. Sirolimus increases transforming growth factor-1 expression and potentiates chronic cyclosporine nephrotoxicity. Kidney Int 2004; 65: 1262-71.
6. Nielsen FT, Ottosen P, Starklint H, Dieperink H. Kidney function and morphology after short-term combination therapy with cyclosporine A, tacrolimus and sirolimus in the rat. Nephrol Dial Transplant 2003; 18: 491-6.
7. Lloberas N, Torras J, Alperovich G, Cruz ado JM, Gíménez-Bonafé P, Herrero-Fresneda I, Franquesa M, Rama J, Grinyó JM. Different renal toxicity profiles in the association of cyclosporine and tacrolimus with sirolimus in rats. Nephrol Dial Transplant 2006; 23: 3111-9.
8. Kahan BD. The synergistic effects of cyclosporine and sirolimus. Transplantation 1997; 63: 170.
9. Schena FP, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC, Campistol JM, Racusen L, Polinsky MS, Goldberg-Alberts R, Li H, Scarola J, Neylan JF; Sirolimus CONVERT Trial Study Group. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. Transplantation 2009; 87: 233-42.
10. Oberbauer R. Protocol conversion from a calcineurin inhibitor based therapy to sirolimus. Transplantation 2009; 87: 57-10.
11. Kim JY, Lim SW, Li C, Kim JS, Ahn KO, Yang HI, Choi BS, Kim YS, Kim J, Bang BK, Yang CW. Effect of FTY720 on chronic cyclosporine nephropathy in rats. Transplantation 2005; 80: 1323-30.
12. Song HK, Han DH, Song JH, Ghee JY, Piao SG, Kim SH, Yoon HE, Li C, Kim J, Yang CW. Influence of sirolimus on cyclosporine-induced pancreas islet dysfunction in rats. Am J Transplant 2009; 9: 2024-33.
13. Ghee JY, Han DH, Song HK, Kim WY, Kim SH, Yoon HE, Choi BS, Kim YS, Kim J, Yang CW. The role of macrophage in the pathogenesis of chronic cyclosporine-induced nephropathy. Nephrol Dial Transplant 2008; 23: 4061-9.
14. Shihab FS, Anodoh TF, Tanner AM, Yi H, Bennett WM. Expression of apoptosis regulatory genes in chronic cyclosporine nephrotoxicity favors apoptosis. Kidney Int 1999; 56: 2147-59.
15. Giachelli C, Bae N, Lombardi D, Majesky M, Schwartz S. Molecular cloning and characterization of 2B7, a rat mRNA which distinguishes smooth muscle cell phenotypes in vitro and is identical to osteopontin (secreted phosphoprotein I, 2aR). Biochem Biophys Res Commun 1991; 177: 867-73.
16. Li C, Yang CW, Ahn HJ, Kim WY, Park CW, Park JH, Cha JH, Kim J, Kim YS, Bang BK. Colchicine suppresses osteopontin expression and inflammatory cell infiltration in chronic cyclosporine nephrotoxicity. Nephron 2002; 92: 422-30.

17. Li C, Lim SW, Sun BK, Choi BS, Glowacka S, Cox A, Kelly D, Kim YS, Kim J, Bang BK, Yang CW. Expression of apoptosis-related factors in chronic cyclosporine nephrotoxicity after cyclosporine withdrawal. Acta Pharmacol Sin 2004; 25: 401-11.

18. Han SW, Li C, Ahn KO, Lim SW, Song HG, Jang YS, Cho YM, Jang YM, Ghee JY, Kim JY, Kim SH, Kim J, Kwon OJ, Yang CW. Prolonged endoplasmic reticulum stress induces apoptotic cell death in an experimental model of chronic cyclosporine nephropathy. Am J Nephrol 2008; 28: 707-14.

19. Ahn KO, Li C, Lim SW, Song HK, Ghee JY, Kim SH, Kim JY, Yoon HE, Cha JH, Kim J, Yang CW. Infiltration of nestin-expressing cells in interstitial fibrosis in chronic cyclosporine nephropathy. Transplantation 2008; 86: 571-7.

20. Yoon HE, Yang CW. Established and newly proposed mechanisms of chronic cyclosporine nephropathy. Korean J Intern Med 2009; 24: 81-92.