

Full Length Research Paper

# Synergistic therapy of enalapril and *Cordyceps sinensis* in the improvement of renal function in chronic allograft nephropathy patient

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Accepted 30 March, 2012

**Chronic allograft nephropathy (CAN) still remains an important factor that affects the long-term survival of renal recipients. The aim of the study was to investigate synergistic effect of enalapril (an angiotensin converting enzyme inhibitor, ACEI) and *Cordyceps sinensis* (Bailing capsule, fermented agent of *C. sinensis*) on CAN and pursue an effective therapy to control CAN progression. A total of 84 CAN patients who underwent transplantation (live related donor, no prisoners were used in this study) were involved in the study and randomized into four groups. Group A (n=22) received combined treatment of enalapril (10 mg/day) and *C. sinensis* (2.0 g/per times, three times per day), group B (n=20) was treated with enalapril (10 mg/day), group C (n=21) with *C. sinensis* (same dose as in group A) and group D (n=21) treated with immunosuppressive agents was set as control. Serum creatinine (SCr), blood urea nitrogen (BUN), creatinine clearance rate (CCr), urinary protein in 24 h (24 h Upro) and urinary transforming growth factor beta 1 (TGF- $\beta_1$ ) of all patients were measured before treatment, and at six months after treatment. After treatment for six months, SCr and CCr were improved while 24 h Upro and urinary TGF- $\beta_1$  decreased in group A, and SCr improved and 24 h Upro decreased in group C. Patients of group A obtained the highest degree of improvement, and more patients obtained renal improvement and stability than in the other groups. The results of the study show that combined use of enalapril and *C. sinensis* takes advantages of reducing excretion of urinary protein, improving renal function and retarding CAN progression for CAN patients compared with single use of enalapril or *C. sinensis*.**

**Key words:** Chronic allograft nephropathy, renal transplantation, enalapril, *Cordyceps sinensis*.

## INTRODUCTION

Clinical use of new immunosuppressive agents significantly improved the short-term outcome of renal transplantation. Nevertheless, chronic allograft nephropathy (CAN) still remains an important factor that affects the long-term survival of renal recipients (Brian and Dirk,

2011). Although the pathogen of CAN is unclear, many immune and/or non-immune factors were risk ones for CAN (Jeffery et al., 2009). Immune and non-immune factors ultimately induced extracellular stromal sedimentation and renal interstitial fibrosis, which was related to excessive secretion of transforming growth factor beta 1 (TGF- $\beta_1$ ) and glomerular lesions in transplanted kidneys (Maristela et al., 2007; Solez et al., 1996). Due to the merits of low toxicity and rare complications, traditional Chinese medicines (TCMs) have been

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**Table 1.** Demographics of the patients with kidney transplantation

Parameter	Group A (n=22)	Group B (n=20)	Group C (n=21)	Group D (n=21)
Recipient age (year)	39.1±11.5	41.2±12.4	38.9±14.3	40.7±13.6
Gender (F/M)	11/11	9/11	9/12	12/9
Donor age (year)	30.3±7.5	28.7±5.2	31.1±6.6	29.2±7.7
Cold-ischemin time(h)	7.7±4.2	7.5±4.3	7.5±4.5	7.6±3.9
HLA-A, B mismatches	2.3±0.8	2.3±0.7	2.4±0.6	2.3±0.6
HLA-DR mismatches	1.2±0.4	1.3±0.7	1.3±0.4	1.2±0.2
Live related donor	22	20	21	21
FK506/CsA	8/14	6/14	9/12	8/13
MMF/Aza	10/12	11/9	12/9	12/9

**Table 2.** Comparison of renal function before and after treatment ( $\bar{x} \pm s$ ).

Group	Time	SCr ( $\mu\text{mol/L}$ )	CCr (ml/min)	BUN (mmol/L)
Group A (n=22)	Before	315.23±43.14	26.79±9.71	16.76±5.06
	6 months	236.82±29.57*	30.88±10.02*	14.44±5.25
Group B (n=20)	Before	296.83±40.10	26.68±11.04	15.65±4.47
	6 months	305.72±37.81	29.32±11.76	15.54±4.69
Group C (n=21)	Before	311.92±41.65	25.98±10.12	16.61±4.75
	6 months	240.06±31.18*	27.44±9.35	14.56±5.65
Group D (n=21)	Before	308.54±39.64	27.10±9.97	15.30±4.53
	6 months	323.25±37.23	25.67±10.21	16.12±5.48

\* P&lt;0.05

D. Urinary TGF- $\beta_1$  was decreased in patients of group A but showed no change in patients of groups B, C or D (Table 3).

### Clinical outcomes

Six months after treatment, renal function was improved at rates of 40.9% in group A, 30.0% in group B, 33.3% in group C, and 14.2% in group D respectively. Renal function was stable at rates of 40.9% in group A, 30.0% in group B, 28.6% in group C and 42.9% in group D, respectively (Table 4).

In addition, no acute graft rejection, infection, hypo-proteinemia or hyperlipemia, impairment of liver function or reduction of white blood cells were observed in all the patients.

### DISCUSSION

Kidney allograft half-life became a focus due to shortage of donor kidneys. Kidney allograft half-life was 7 to 10

years; longer than that reported in China Nidyanandh et al., 2007). Progressive failure in kidney function occurred in many patients a few years or even a few months after renal transplantation, mainly due to CAN. Although pathogenic mechanism for CAN was not clear, ischemia-reperfusion injury, graft rejection, cytomegalovirus infection, and renal toxicity of CsA were proved to be the main causes for CAN (Citterio et al., 2004; Scherer et al., 2003). The main pathological change of CAN was renal interstitial fibrosis and tubular atrophy, together with decrease in GFR, hypertension, and proteinuria, which resulted in increase of SCr and loss of transplanted kidneys ultimately (Freese et al., 2001). CAN was controlled mainly through adjustment of immunosuppressive agents, restriction of protein intake, and treatment of hypertension. Nevertheless, no drug reported can treat CAN effectively at present (Weir et al., 1998; Campistol et al., 1999).

The primary pathology of CAN was renal interstitial fibrosis. Recent studies indicated that TGF- $\beta_1$  played a key role in interstitial fibrosis (Woo et al., 2003). Animal experiment proved that recombinant TGF- $\beta_1$  induced glomerular sclerosis, and inhibitor of TGF- $\beta_1$  could retard

**Table 3.** Comparison of 24 h Upro and urinary TGF- $\beta_1$  before and after treatment ( $\bar{x} \pm s$ ).

Group	Time	24 h Upro (g/24 h)	UrineTGF- $\beta_1$ (pg/mg. Cr)
Group A (n=22)	Before	2.12 $\pm$ 0.71	468.38 $\pm$ 121.17
	6 months	1.33 $\pm$ 0.29*	274.45 $\pm$ 65.06*
Group B (n=20)	Before	1.98 $\pm$ 0.63	448.74 $\pm$ 127.31
	6 months	2.06 $\pm$ 0.65	379.92 $\pm$ 86.36
Group C (n=21)	Before	1.96 $\pm$ 0.87	457.61 $\pm$ 118.30
	6 months	1.24 $\pm$ 0.32*	421.22 $\pm$ 90.46
Group D (n=21)	Before	2.02 $\pm$ 0.76	436.87 $\pm$ 123.82
	6 months	2.31 $\pm$ 0.58	478.54 $\pm$ 105.33

\* P&lt;0.05.

**Table 4.** Comparison of clinical outcomes among different groups.

Group	Improved (%)	Stabilized (%)	Worsened (%)
Group A (n=22)	9 (40.9%)	9 (40.9%)	4 (18.2%)
Group B (n=20)	6 (30.0%)	6 (30.0%)	8 (40.0%)
Group C (n=21)	7 (33.3%)	6 (28.6%)	8 (38.1%)
Group D (n=21)	3 (14.2%)	9 (42.9%)	9 (42.9%)

renal fibrosis (Amann et al., 2001; Houlihan et al., 2002). Since TGF- $\beta_1$  production was regulated by rennin-angiotensin system, inhibitor of angiotensin II receptor or inhibitor of angiotensin II converting enzyme would have effect on CAN (Radermacher et al., 2003). Prospective study revealed that these agents reduced plasma TGF- $\beta_1$  in CAN patients (Attila et al., 2000). In this study, compared with groups B and C, CCr was increased and urinary TGF- $\beta_1$  decreased in group A after treatment with *C. sinensis* and enalapril for six months, and considerable number of patients acquired improvement or stabilization of renal function. Our results indicate that combined use of *C. sinensis* and enalapril exerts protective effect on transplanted kidneys. There was report that TGF- $\beta_1$  mRNA was significantly decreased in CAN patents treated with ACEI (Wang et al., 2005). It was possible that enalapril protected renal tubule and reduced TGF- $\beta_1$  secretion, which resulted in improvement or stabilization of renal function. Nevertheless, CCr, BUN, and 24 h Upro were not improved in patients of group B, and no parameters for renal function were improved after treatment for six months. Simple use of enalapril in a short period of time (six months) showed no effect on improvement of renal function of CAN patients.

As a fermented agent of *C. sinensis*, Bailing capsule was proven to contribute to functional improvement or stabilization of the transplanted kidneys (Lu, 2002). *C. sinensis* could inhibit proliferation of mesangium cells and compensatory hypertrophy of glomerulus. *C. sinensis* could also retard and decrease rupture of lysosome membrane of renal tubules, and diminish lipid

peroxidation. In addition, *C. sinensis* could promote proliferation and restoration of renal tubular cells (Kahan et al., 2003). 24 h Upro significantly decreased in patients of group C after *C. sinensis* treatment for six months. Our results suggest that *C. sinensis* attenuated renal tubular lesions. *C. sinensis* activated macrophages and lymphocytes outside the reticuloendothelial systems and the parenchymal organs. It could selectively suppress immunity of parenchymal organs but did not reduce systematic immune function. *C. sinensis* also increase serum IgG through regulation of humoral immunity, which resulted in lower infection and improvement of acratia and edema (Kahan et al., 2003; Sun et al., 2004). After *C. sinensis* treatment for six months, SCr decreased significantly in patients of group C. Renal function was improved in seven patients and was stable in six patients in group C. It was possible that *C. sinensis* had activity of immune regulation and renal protection.

The numbers of patients that acquired improvement and stabilization of the renal function were comparable in groups B and C. Significant decrease of SCr and 24 h Upro was observed in group C after treatment for six months, without change of urinary TGF- $\beta_1$ . However, CCr was increased and urinary TGF- $\beta_1$  decreased in group A after treatment for six months, which may be that *C. sinensis* could reinforce the role of enalapril in reducing urinary TGF- $\beta_1$  and retarding CAN progression. The transplanted kidneys may be worsened in a short time when SCr was higher than 350  $\mu$ mol/L, which was a common experience in clinical practice. It was therefore important to restore renal function as early as possible.

*C. sinensis* could reduce urinary protein more effectively. SCr, CCr, 24 hUpro, and urinary TGF- $\beta_1$  improved earlier and to a greater extent in patients of group A. In addition, more patients in this group acquired functional improvement and stabilization of transplanted kidneys. We hypothesized that enalapril and *C. sinensis* exhibited synergistic effect through regulating immunity and reducing TGF- $\beta_1$  production. No cross reaction or serious side effects were observed in our study.

CAN was treated mainly through adjustment or replacement of immunosuppressive agents, which had limited effect of guarding against loss of transplanted kidneys (Morales et al., 2001; Jeremy et al., 2005). Our results show that combined use of enalapril and *C. sinensis* could reduce urinary protein in CAN patients and retard CAN progression. Combined therapy was superior to enalapril or *C. sinensis* alone in the treatment of CAN. Therefore, combined use of enalapril and *C. sinensis* is recommended for the treatment of CAN, together with adjustment or replacement of immunosuppressive agents.

## ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (No. 81172443) and the Medical Technology Project of Chongqing Municipal Health Bureau (No.2009C205).

## REFERENCES

- Amann K, Koch A, Hofstetter J, Gross ML, Haas C, Orth SR, Ehmke H, Rump LC, Ritz E (2001). Glomerulosclerosis and progression: effect of subantihypertensive doses of alpha and betablockers. *Kidney Int.* 60(4): 1309-1323.
- Attila S, Jens L, Karina S, Hamar P, Philipp T, Heemann U (2000). Effect of angiotensin-converting enzyme inhibition on growth factor mRNA in chronic renal allograft rejection in the rat. *Int. Soc. Nephrol.* 57(3): 982-991.
- Brian JN, Dirk RJ (2011). Diagnosis and prevention of chronic kidney allograft loss *Lancet.* 378: 1428-1437.
- Campistol M, Inigo P, Jimenez W, Lario S, Clesca PH, Oppenheimer F, Rivera F (1999). Losartan decreases plasma levels of TGF- $\beta_1$  in transplant patients with chronic allograft nephropathy. *Kidney Int.* 56(2): 714-719.
- Citterio F, Pozzetto U, Romagnoli J, Tondolo E, Silvestri P, Nanni G, Castagneto M (2004). Plasma levels of transforming growth factor- $\beta_1$  in renal transplant recipients receiving different immunosuppressive regimens. *Transplant Proc.* 36(3): 698-699.
- Freese P, Svalander CT, Molne J, Gunnela N, Gudrun N (2001). Chronic allograft nephropathy: biopsy findings and outcome. *Nephrol. Dial Transpl.* 16(12): 2401-2406.
- Houlihan CA, Akdeniz A, Tsalamandris C, Cooper ME, Jerums G, Gilbert RE (2002). Urinary transforming growth factor-beta excretion in patients with hypertension, type 2 diabetes, and elevated albumin excretion rate: effects of angiotensin receptor blockade and sodium restriction. *Diabetes Care.* 25(6): 1072-1077.
- Huang L, Liang Y, Guo F, Zhou Z, Cheng B (2003). Simultaneous separation and determination of active components in *Cordyceps sinensis* and *Cordyceps militaris* by LC/ESI-MS. *J. Pharm. Biomed. Anal.* 33(5): 1155-1162.
- Jeffery TF, Brian JN, Stephen IA (2009). Chronic allograft nephropathy. *Pediatr Nephrol.* 24(8): 1465-1471.
- Jeremy R, Philip J, Connell O, Brian JN (2005). Chronic renal allograft dysfunction. *J. Am. Soc. Nephrol.* 16: 3015-3026.
- Kahan BD, Karlix JL, Ferguson RM, Garcia R, Skerjanec RA, Schmouder H, Tedesco-Silva J, Medina-Pestana JO (2003). Pharmacodynamics, pharmacokinetics, and safety of multiple doses of FTY720 in stable renal transplant patients: a multicenter, randomized, placebo-controlled, phase I study. *Transpl.* 76(7): 1079-1084.
- Lu L (2002). Study on effect of *cordyceps sinensis* and artemisinin in preventing recurrence of lupus nephritis. *Chin. J. Integr. Tradit. West Med.* 22(3): 169-171.
- Maristela LO, Akihiro T, Naohiko K, Atsuo G, Hiroaki M, Toshiro F (2007). Dual blockade of aldosterone and angiotensin II additively suppresses TGF- $\beta$  and NADPH oxidase in the hypertensive kidney. *Nephrol. Dialysis Transpl.* 22(5): 1314-1322.
- Morales M, Andres A, Rengel M, Rodicio JL (2001). Influence of cyclosporin, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. *Nephrol. Dial Transpl.* 16: 121-124.
- Nidyanandh V, Stefan G, Tullius, Anil C (2007). Chronic Allograft Nephropathy. *Seminars Nephrol.* 27(4): 414-429.
- Racusen LC, Solez K, Colvin RB, Robert B (1999). The Banff97 working classification of renal allograft pathology. *Kidney Int.* 55: 713-723.
- Radermacher J, Mengel M, Ellis S, Stephan S, Markus H, Schwarz A, Eisenberger U, Michael B, Friedrich C, Wilfried G, Haller H (2003). The renal arterial resistance index and renal allograft survival. *N. Engl. J. Med.* 349: 115-124.
- Scherer A, Krause A, Walker JR, Korn A, Niese D, Raulf F (2003). Early prognosis of the development of renal chronic allograft rejection by gene expression profiling of human protocol biopsies. *Transpl.* 75(8): 1323-1330.
- Solez K, Benediktsson H, Cavallo T, Croker B, Demetris AJ, Drachenberg C, Yilmaz S (1996). Report of the Third Banff Conference on Allograft Pathology (July 20-24, 1995) on classification and lesion scoring in renal allograft pathology. *Transplant Proc.* 28(1): 441-444.
- Sun M, Yang YR, Lu YP, Gao R, Wang L, Wang J, Tang K (2004). Clinical study on application of *Cordyceps sinensis* after renal transplantation. *Chin. J. Integr. Tradit West Med.* 24(9): 808-810.
- Wang PX, Jia WS, Feng JY (2005). Clinical investigation of enalapril's effect on early chronic allograft nephropathy. *J. Nephrol. Dialy Transpl.* 14(1): 18-22.
- Weir MR, Schweitzer EJ, Yoon S, Wiland A, Anderson L, Kuo PC, Lim JW, Johnson LB, Farney AC, Weir MR, Bartlett ST (1998). Mycophenolate mofetil reduces the risk of acute rejection less in African-American than in caucasina kidney recipients. *Transpl.* 65(2): 242-248.
- Woo YC, Jong AH, Young KW (2003). Synergistic effects of mycophenolate mofetil and enalaprilin a model of chronic cyclosporine nephropathy. *Transpl.* 75(2): 309-315.