African Journal of Biotechnology Vol. 11(32), pp. 8149-8153, 19 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.449
ISSN 1684–5315 © 2012 Academic Journals

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-ß$_1$) 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-ß$_1$ 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-ß$_1$) 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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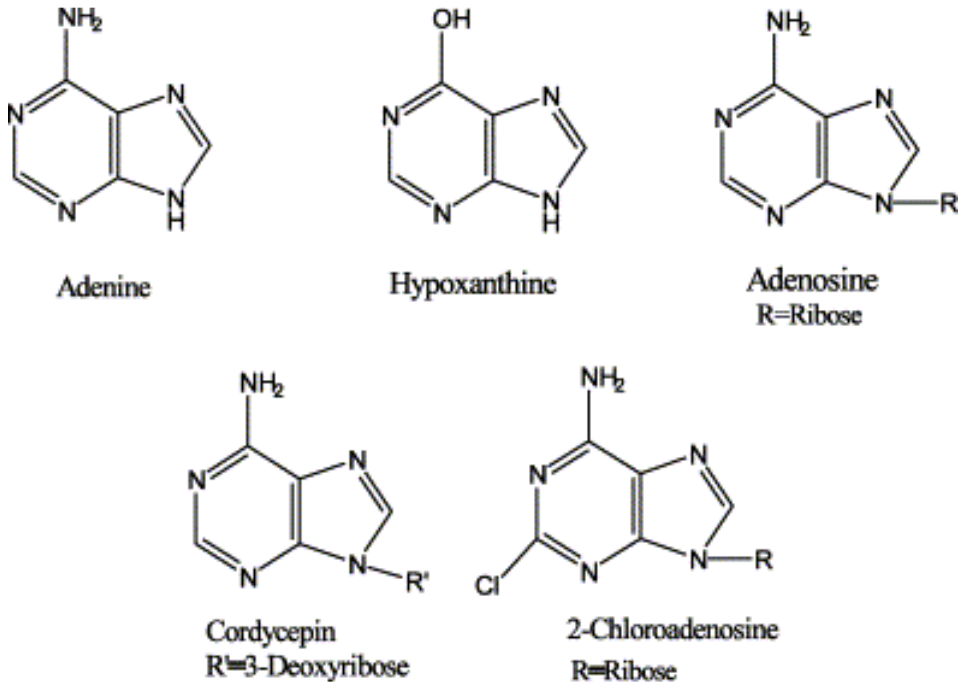

Figure 1. Molecular structures of adenine, hypoxanthine, adenosine, cordycepin and 2-chloroadenosine.

extensively used as herbal medicine to prevent and cure many diseases for over a millennium. It has been demonstrated that adenine, hypoxanthine, adenosine and cordycepin are the major bioactive components in *C. sinensis* (Huang et al., 2003). Their molecular structures are shown in Figure 1. In this study, we treated CAN patients with enalapril (an angiotensin converting enzyme inhibitor, ACEI) and BAILING capsule (fermented agent of *C. sinensis*) and investigated their synergistic effect on improvement of renal function and control of CAN progression.

MATERIALS AND METHODS

A total of 41 male and 43 female patients with chronic allograft nephropathy (CAN) who underwent transplantation between 2006 and 2008 were investigated. The age of the patients was 34.3±15.5 years, ranging from 21 to 71 years. CAN was diagnosed by renal biopsy and histological examination (Racusen et al., 1999). All patients underwent renal transplant of living related donor (no prisoners were used in this study). Renal dysfunction occurred at 10 to 87 months (38.6±27.7) after operation. Additional examinations were performed to exclude acute graft rejection, recurrent glomerulonepheritis, obstruction/reflux, vascular stenosis of transplanted kidney, CsA toxicity, etc.

The patients were randomly divided into four groups, that is, enalapril and *C. sinensis* group (group A, n=22), enalapril group (group B, n=20), *C. sinensis* group (croup C, n=21) and control group (group D, n=21). All the patients received treatment with cyclosporine A (CsA), tacrolimus (FK506), azathioprine (Az), mycophenolate mofetil (MMF) and prednisolone (Pred). The primary doses of CsA and FK506 were 6 and 0.15 mg/kg per day respectively, and doses were adjusted according to drug concentration in blood. There was no significant difference among groups in gender, age, tissue matching, cold ischemia length, blood pressure, blood lipid, survival length, initial serum creatinine (SCr), blood urea nitrogen (BUN), creatinine clearance rate (CCr), urine protein in 24 h (24 h Upro) and TGF- $ß_1$ at the beginning of investigation (Table 1). In addition to immunosuppressive treatment, patents in group A received combined treatment of enalapril (10 mg/day) and *C. sinensis* (2.0 g/per times, three times per day), patients in group B received enalapril only (10 mg/day), patients in group C received *C. sinensis* only (same dose as in group A), and patients in group D still received immunosuppressive treatment. SCr, BUN, CCr and 24 h Upro of all patients were measured before and at six months after treatment. Urinary TGF-$ß_1$ was also measured by enzyme-linked immunosorbent assay (ELISA). To avoid dilution effects, the ratio of the measured urinary value of TGF- $ß_1$ to urinary creatinine was used.

Six months after treatment, renal function was assessed to be improved if SCr was decreased or CCr increased by 20%. On the other hand, renal function was assessed to be worsened if SCr was increased or CCr decreased by 20%. Renal function was assessed to be stable if the change of SCr or CCr was lower than 20%. The data were analyzed with SPSS11.0 and expressed by mean±standard deviation. $\chi 2$ test, *t* test and *F* test were performed. $P<0.05$ was considered statistical significant.

RESULTS

Parameters for renal function

SCr was decreased and CCr increased in patients of group A after treatment for six months ($P<0.05$). SCr was decreased ($P<0.05$) in group C, whereas CCr did not change. Neither SCr nor CCr was significantly improved in patients of groups B and D as shown in Table 2

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 ($\bar{x}$ ±s).

Group	Time	SCr (μmol/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<0.05

D. Urinary TGF-ß$_1$ 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 immuno-suppressive 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-ß$_1$ played a key role in interstitial fibrosis (Woo et al., 2003). Animal experiment proved that recombinant TGF-ß$_1$ induced glomerular sclerosis, and inhibitor of TGF-ß$_1$ could retard

Table 3. Comparison of 24 h Upro and urinary TGF-ß1 before and after treatment ($\bar{x}$ ±s).

Group	Time	24 h Upro (g/24 h)	UrineTGF-ß1 (pg/mg. Cr)
Group A (n=22)	Before	2.12±0.71	468.38±121.17
	6 months	1.33±0.29*	274.45±65.06*
Group B (n=20)	Before	1.98±0.63	448.74±127.31
	6 months	2.06±0.65	379.92±86.36
Group C (n=21)	Before	1.96±0.87	457.61±118.30
	6 months	1.24±0.32*	421.22±90.46
Group D (n=21)	Before	2.02±0.76	436.87±123.82
	6 months	2.31±0.58	478.54±105.33

* P<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-$ß_1$ 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-$ß_1$ in CAN patients (Attila et al., 2000). In this study, compared with groups B and C, CCr was increased and urinary TGF-$ß_1$ 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-$ß_1$ 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-$ß_1$ 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 wasobserved in group C after treatment for six months, without change of urinary TGF-$ß_1$. However, CCr was increased and urinary TGF-$ß_1$ 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-$ß_1$ and retarding CAN progression. The transplanted kidneys may be worsened in a short time when SCr was higher than 350 µmol/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-ß$_1$ 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-ß$_1$ 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 wa supported by the National Natural Science Foundation of China (No. 81172443) and the Medical Technology Project of Chongqing Municipal Health Bureau (No.2009C205).

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