Sairei-to enhances the antinephrotic effect of cyclosporine A in puromycin aminonucleoside-treated rats

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Abstract

Sairei-to (TJ-114), a traditional herbal medicine, has been used clinically as therapy for nephrotic syndrome. This experiment was conducted to clarify whether TJ-114 could enhance the antinephrotic effect of cyclosporine A (CyA). Nephrosis was induced in rats by a single intravenous injection of puromycin aminonucleoside (PAN). TJ-114 and CyA were orally administered consecutively to the nephrotic rats. In PAN-treated rats, urinary protein excretion and levels of cholesterol and triglyceride in serum were increased, whereas the serum albumin level was decreased. Treatment with CyA dose-dependently attenuated these changes, and TJ-114 also inhibited the induction of nephrosis in the PAN-treated rats. Furthermore, it has been demonstrated that the concomitant use of TJ-114 with CyA enhanced the antinephrotic effect at a dose of CyA 10mg/kg, and conversely inhibited the increase in serum creatinine at a dose of CyA 40mg/kg in nephrotic rats. These findings suggested that TJ-114 may be useful for lowering the therapeutic dose of CyA in nephrotic syndrome.

Key words Sairei-to, cyclosporine A, aminonucleoside, nephrosis.

Abbreviations CyA, cyclosporine A ; PAN, puromycin aminonucleoside ; Sairei-to (Chai-Ling-Tang, 柴苓湯).

Introduction

Sairei-to (TJ-114), a traditional herbal medicine, has been used clinically as therapy for nephrotic syndrome and glomerular nephritis.[1] Yoshikawa *et al.*[2] reported that TJ-114 reduced urinary protein excretion in children with IgA nephropathy showing focal/minimal mesangial proliferation in a prospective controlled study. Furthermore, combined administration of TJ-114 with steroids for nephrotic syndrome has been useful for reducing the steroidal dose or lowering the recurrence rate.[3]

Cyclosporine A (CyA), an immunosuppressant, is useful for the treatment of nephrotic syndrome in cases resistant to steroid therapy or demonstrating frequent relapses. It has been reported that CyA promotes the synthesis of proteoglycans by glomerular epithelial cells due to its inhibitory effects on the production of glomerular platelet-activating factor (PAF) and tumor necrosis factor-alpha (TNF-alpha) in PAN nephrosis.[4] However, CyA has various side effects involving nephrotoxicity,[5] and the recurrence rate of nephrosis after discontinuation of CyA therapy is high. Hence, a decrease in the therapeutic dose of CyA is expected to attenuate the nephrotoxicity of CyA and allow CyA therapy to be continued.

This study evaluated whether TJ-114 could enhance the antinephrotic effect of CyA in PAN-treated rats, and thereby clarify the possibility of decreasing the therapeutic dose of CyA for nephrosis.

Materials and Methods

Experimental animals : Male Sprague-Dawley rats weighing 180-200g were used. The animals were kept at 23℃ on a 12hr light-dark cycle. They had free access to

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tap water and commercial chow.

Drugs : TJ-114 is composed of 12 crude drugs in fixed proportions: Bupleuri radix 7.0g, Pinelliae tuber 5.0g, Alismatis rhizoma 4.0g, Scutellariae radix 3.0g, Ginseng radix 3.0g, Zizyphi fructus 3.0g, Polyporus 3.0g, Atractylodis lancea rhizoma 3.0g, Hoelen 3.0g, Glycyrrhizae radix 2.0g, Cinnamomi cortex 1.5g and Zingiberis siccatum rhizoma 1.0g. The drug was prepared as a spray-dried powder from a hot-water extract. CyA was purchased from Novartis Phama Co. PAN was purchased from Sigma Chemical Co.

Experimental protocol : Nephrosis was induced in rats by a single intravenous injection of PAN (100mg/kg). Animals injected with physiological saline (4mL/kg, i.v.) served as normal controls. TJ-114 (1.0g/kg) was administered orally once a day starting just after the injection of PAN. CyA (10, 20, 40mg/kg/day) was suspended in 1% Tween 80 and administered orally to rats from 6 days after PAN injection in order to avoid nephrotoxicity of CyA. Nephrotic severity was assessed by urinary protein excretion 6, 11 and 15 days after PAN treatment, and the levels of serum cholesterol, triglyceride and albumin were measured 15 days after the treatment.

Urinary protein excretion and serum parameters: Each group of rats was raised in a metabolic cage and given tap water *ad libitum*. Twenty four hour urine specimens were collected periodically. Urinary protein was determined by the dye-binding assay (Tonein TP-2, Otsuka Pharmaceutical Co., Japan). Blood was collected from the portal vein. Serum cholesterol, triglyceride and creatinine concentrations were determined by enzymatic methods, and serum albumin was assayed by the bromocresol purple method with an auto analyzer (Toshiba 20FR, Tokyo Japan).

Statistics : The significance of differences among values of individual parameters was evaluated using Fisher's test. All values are expressed as the mean±SE.

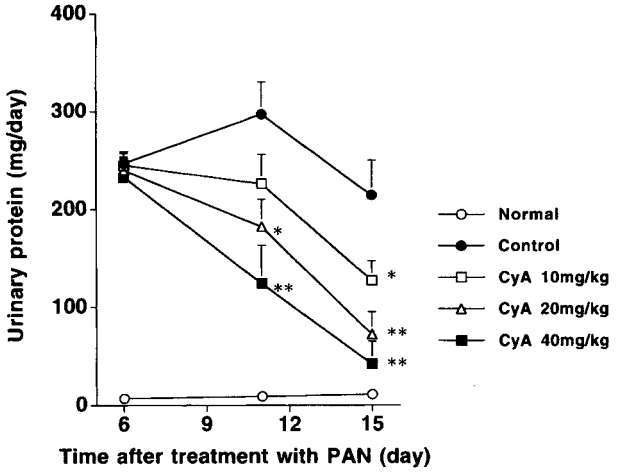

Fig. 1 Effect of cyclosporine A (CyA) on urinary protein excretion in rats with PAN-induced nephrosis. All values expressed as the mean ±SE (n=5).
*p<0.05, **P<0.01 compared to control

Results

Antinephrotic effect of CyA in PAN-treated rats

The level of urinary protein excretion in rats with PAN-induced nephrosis was dose-dependently reduced by continuous administration of CyA (Fig.1). The levels of serum cholesterol, triglyceride and albumin in the nephrotic rats were barely influenced by treatment with 10mg/kg of CyA, but were improved by treatment with 20 or 40mg/kg of CyA (Table I). In contrast, the decrease in body weight of nephrotic control rats compared to normal rats was significantly enhanced by the administration of CyA (40mg/kg), and the level of serum creatinine in rats administered CyA (40mg/kg) was most

Table 1 Effects of CyA on body weight, serum cholesterol, triglyceride, albumin and creatinine in PAN-induced nephrotic rats

	Body weight (g)	Serum cholesterol (mg/dL)	Serum triglyceride (mg/dL)	Serum albumin (g/dL)	Serum creatinine (mg/dL)
Normal	289.7±8.9	71±3	56±5	1.45±0.02	0.34±0.01
Control	242.8±1.9##	257±40##	276±66##	0.92±0.06##	0.41±0.04#
CyA 10mg/kg	239.1±4.9	189±8 *	202±68	1.06±0.05	0.38±0.02
CyA 20mg/kg	237.6±5.0	158±12**	137±34	1.18±0.06**	0.35±0.01
CyA 40mg/kg	212.9±8.9**	147±24**	196±59	1.19±0.06**	0.46±0.02##

Data were represented the mean±SE (n=5). *p<0.05, **p<0.01 compared to Control, #p<0.05, ##p<0.01 compared to Normal

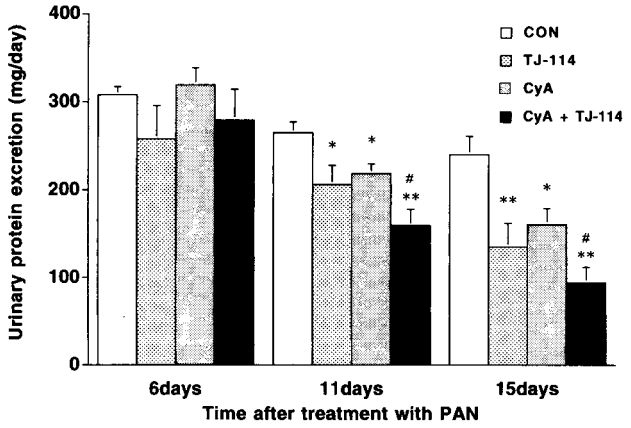

Fig. 2 Effect of TJ-114 (1.0g/kg,p.o.) and CyA (10mg/kg,p.o.) on urinary protein excretion in rats with PAN-induced nephrosis. All values are expressed as the mean ±SE (n=9-10).
*p<0.05, **p<0.01 compared to control (CON), #p<0.05 compared to CyA.

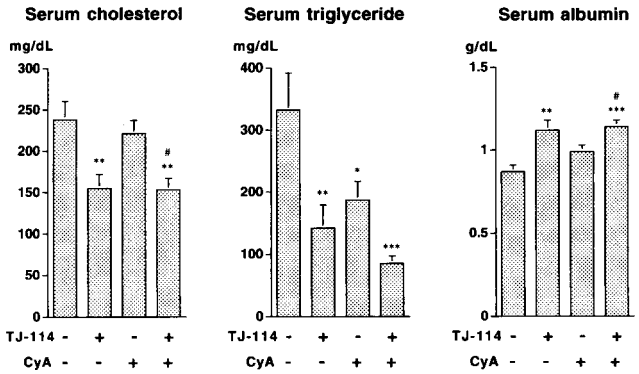

Fig. 3 Effect of TJ-114 (1.0g/kg,p.o.) and CyA (10mg/kg,p.o.) on serum cholesterol, triglyceride and albumin in rats with PAN-induced nephrosis. Data was collected 15 days after PAN treatment. All values are expressed as the mean ±SE (n=9-10).
*p<0.05, **p<0.01, ***p<0.001 compared to control (CON), #p<0.05 compared to CyA.

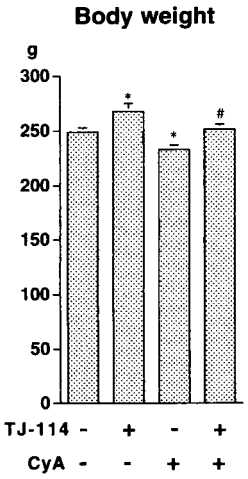

Fig. 4 Effect of TJ-114 (1.0g/kg,p.o.) and CyA (10mg/kg,p.o.) on body weight in rats with PAN-induced nephrosis. Data was collected 15 days after PAN treatment. All values are expressed as the mean ±SE (n=9-10).
*p<0.05, compared to control (CON), #p<0.05 compared to CyA.

elevated of all the groups.

Influence of TJ-114 on the antinephrotic effect of CyA

In the rats administered TJ-114, urinary protein was not changed compared to nephrotic control rats 6 days after PAN treatment, but was significantly reduced by about 20% at 11 days and by about 45% at 15 days (Fig.2). The concomitant use of TJ-114 and CyA at 10mg/kg most inhibited urinary protein excretion, with a significant decrease compared to CyA alone. Furthermore, this concomitant use was more effective against serum triglyceride than a single treatment 15 days after the PAN treatment, and also improved the abnormalities of serum cholesterol and albumin, however the efficacy was as good as that of TJ-114 alone (Fig.3). Body weight was significantly decreased by treatment with CyA alone, but not in the concomitant use of TJ-114 and CyA (Fig.4).

In CyA (40mg/kg)-treated rats with PAN-induced nephrosis, TJ-114 (1.5g/kg) did not influence the body weight (212.9±8.9g in control vs 204.3±6.0g in TJ-114) , however it significantly improved the increase in serum creatinine (0.46±0.02mg/dL in control vs 0.38 ±0.01mg/dL in TJ-114; p<0.05).

Discussion

In the present study, it was clarified that Sairei-to (TJ-114) enhanced the inhibitory effects of CyA on proteinuria when administered in combination with a semi-antinephrotic dose (10mg/kg) of CyA in PAN-treated rats. It has been reported that TJ-114 prevented proteinuria in PAN-induced nephrosis in rats,[6] and anti-glomerular basement membrane (GBM) nephritic rats.[7,8] In this study, it was also demonstrated that TJ-114 decreased urinary protein excretion in PAN-treated rats. This result confirmed that TJ-114 was effective against nephrosis. In addition, it was shown that the concomitant use of TJ-114 and CyA was more effective than a single treatment in these models.

Although the mechanism of the antinephrotic effect of TJ-114 has not been defined, it has been reported that TJ-114 increases corticosterone secretion by stimulating the pituitary-adrenal system.[9] Steroids, which have been the most appropriate initial treatment for patients with nephrotic syndrome, were demonstrated to ameliorate urinary protein excretion in rats with PAN-induced

nephrosis, and this mechanism was considered to inhibit decreases in the proteoglycan concentration in the glomerular basement membrane.[10] These findings suggest that the antinephrotic effect of TJ-114 is due to an endogenous steroid-stimulating action.

Moreover, it has been reported that TJ-114 enhanced the scavenging of reactive oxygen species in rats with PAN-induced nephrosis.[11] It is considered that reactive oxygen species are involved in the pathophysiology of PAN-induced nephrosis.[12] Aoyagi *et al.*[13] reported that PAN activated protein kinase C resulting in an increase in the hydroxyl radical in isolated rat hepatocytes. In addition, oxidative stress was observed in patients with nephrotic syndrome. It has been shown that saikosaponins including Bupleuri radix extract and baicalein including Scutellariae radix extract among the components of TJ-114 markedly inhibited the generation of reactive oxygen species.[14-16] Saikosaponin-d has been reported to prevent the development of proteinuria induced by PAN in rats.[17] These reports suggest that the antioxidant effect of TJ-114 is partially related to the amelioration of nephrosis.

Thereby, it was suggested that TJ-114 inhibited the injury of glomerular basement membrane by the endogenous steroid-stimulating and antioxidant action in rats with PAN-induced nephrosis. Since it has been reported that CyA promotes the synthesis of proteoglycans by glomerular epithelial cells, it is considered that these actions of TJ-114 may play roles in accelerating that CyA repair the damage of glomerular basement membrane in rats with PAN-induced nephrosis.

In this experiment, the concomitant use of TJ-114 and CyA attenuated the abnormalities of serum parameters 15 days after PAN treatment, however the efficacy against serum cholesterol and albumin was as good as that of TJ-114 alone. These findings may be attributed to the action of a semi-antinephrotic dose (10mg/kg) of CyA, which was insufficient for the improvement of serum abnormalities, but not proteinuria in PAN-induced nephrosis in rats.

CyA has various side effects in volving nephrotoxicity. In this study, body weight was decreased by treatment with CyA in rats with PAN-induced nephrosis. However, the concomitant use of TJ-114 and CyA at 10mg/kg created no changes in body wieght. Furthermore, this study demonstrated that TJ-114 significantly inhibited the increase in serum creatinine caused by treatment with CyA 40mg/kg in rats with PAN-induced nephrosis. These results suggested that TJ-114 reduced the adverse effect of CyA, nevertheless it enhanced the antinephrotic effect of CyA.

It is well known that CyA has functional and structural nephrotoxicity, causing an acute decrease in renal cortical and medullary blood flow, a fall in glomerular capillary perfusion, and contraction of mesangial cells. Furthermore, chronic administration of CyA leads to the loss of proximal tubular epithelial cell integrity and tubular atrophy, a variable interstitial injury with secondary fibrosis.[5] Calcium channel blockers,[18] captopril[19] and endothelin antagonists[20] have been employed to counter the effect of CyA, which decreases renal blood flow by constricting the afferent arteriole to the glomerulus. It has been reported that TJ-114 inhibited the synthesis of endothelin-1 in rats with anti-GBM nephritis.[8] The inhibition of the increase in serum creatinine in CyA-treated rats with PAN-induced nephrosis may contribute to the decrease in endothelin-1 synthesis of TJ-114. In addition, it has been suggested that reactive oxygen species play a role in the CyA-mediated impairment of renal function. It was suggested that the antioxidant action of TJ-114 was associated with the enhancement of antinephrotic effect of CyA, but also the reduction the adverse effect of CyA.

In conclusion, TJ-114 enhanced the antinephrotic effect of CyA, however, it inhibited the nephrotoxicity of CyA in PAN-induced nephrosis among rats. These results suggest that TJ-114 may be useful for lowering the therapeutic dose of CyA in the nephrotic syndrome. Further evaluation is necessary to clarify the antinephrotic mechanism and protective effect of TJ-114 against CyA-induced nephrotoxicity.

和文抄録

柴苓湯は臨床の場においてネフローゼ症候群の治療に用いられている。本実験では，柴苓湯がサイクロスポリンA（CyA）のネフローゼに対する作用を増強しうるか否か検討した。ラットにピューロマイシン・アミノヌクレオシド（PAN）を静脈内投与してネフローゼを惹起させ，柴苓湯およびCyAを連日強制経口投与した。PANを投与したラットでは，蛋白尿および血清コレステロールと血清トリグリセリドの増加，血清アルブミンの低下

が見られた。CyAはこれらの変化を用量依存的に減弱し，柴苓湯も改善作用を示した。さらに柴苓湯はCyA (10mg/kg) との併用投与により，CyAの抗ネフローゼ作用を増強させた。一方，CyA (40mg/kg) をネフローゼラットに投与すると血清クレアチニンの増加が見られたが，柴苓湯との併用投与ではこれを抑制した。以上の結果より，柴苓湯はネフローゼ症候群におけるCyAの治療投与量の低下に有用であることが示唆された。

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