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Marked Decrease of Cyclosporin Absorption Caused by Coadministration of *Cordyceps sinensis* in Rats

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ABSTRACT

Cordyceps (*Cordyceps sinensis* SACCARDO.) is a popular traditional tonic used in Taiwan and mainland China. Cyclosporin is an important immunosuppressant with narrow therapeutic index. This study investigated the effect of coadministration of cordyceps on cyclosporin pharmacokinetics in rats. Cyclosporin was intravenously (0.8 mg/kg) and orally (2.5 mg/kg) administered with and without cordyceps (2.0 g/kg), respectively, in crossover designs. Blood samples were collected by cardiopuncture and quantitated by a specific monoclonal fluorescence polarization immunoassay. Pharmacokinetic parameters of cyclosporin were calculated using noncompartment model. Paired Student's *t*-test was used for statistical comparison. Our results indicated that oral coadministration of cordyceps significantly decreased the C_{max} and $AUC_{0\text{-}540}$ of cyclosporin by 85.5% and 83.2%, respectively. In addition, when rats were given cordyceps 1 hr before cyclosporin dosing, the C_{max} and $AUC_{0\text{-}540}$ of cyclosporin were significantly decreased by 85.9% and 84.4%, respectively. By contrast, when cyclosporin was given as an intravenous bolus, the disposition of cyclosporin was not altered by cordyceps, indicating that the interaction between oral cyclosporin and cordyceps occurred during the absorption phase. In conclusion, cordyceps markedly decreased the oral bioavailability of cyclosporin. Patients treated with cyclosporin are suggested to avoid the concurrent use of cordyceps for efficacy and safety.

Key words: *Cordyceps sinensis*, cyclosporin, pharmacokinetics, drug interaction

INTRODUCTION

Cordyceps sinensis SACCARDO. (CS), a fungus parasitic on the insect larvae of *Lepidoptera*, is an important Chinese medicine used as a tonic in Taiwan and mainland China. Traditional uses of CS include treatments for respiratory diseases like asthma, bronchial and lung inflammation, cardiovascular diseases like arrhythmia and hypertension, as well as kidney and liver diseases[1-3]. Recent studies reported various pharmacological effects of cordyceps, including anti-hyperglycemia[4], antioxidation[5-7], inhibition of tumors[8,9], induction of mesangial cell apoptosis[10] and stimulation of steroidogenesis[11,12]. In addition, cordyceps also demonstrated immunomodulatory effect[13] and had been proven beneficial for autoimmune disease[14].

CS was reported to contain various chemical compositions including proteins, amino acids, fats, nucleosides and carbohydrates. The amino acids in CS consist of glutamic acid, aspartic acid, arginine and glycine[15,16]. Nucleosides in CS include adenosine and cordycepin (3'-deoxyadenosine), which have usually been assumed to be the bioactive ingredients and indices for estimation of the CS quality[17]. Polysaccharides are one of the major active ingredients in CS and possess various beneficial effects such as inhibition of lipid peroxidation[5], prevention of hemolysis[6] and inhibition of tumors[7].

In oriental countries, traditional Chinese medicines have been extensively used to prevent and cure many diseases due to low toxicity and rare complication. However, the hidden risks of herb - drug interactions are generally overlooked. Cyclosporin is an important immunosupressant with narrow therapeutic index. Subtherapeutic blood level of cyclosporin causes allograft rejection, whereas supratherapeutic blood level leads to nephrotoxicity, hepatotoxicity or neurotoxicity[18]. This study investigated the effect of coadministration of cordyceps on the pharmacokinetics of cyclosporin in rats.

MATERIALS AND METHODS

I. *Chemicals*

Cyclosporin (Neoral®, 100 mg/mL) was provided by

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Novartis Co. (Taiwan) and properly diluted with water to 1.25 mg/mL. Rhodamine 123 was purchased from Aldrich (Milw. WI, USA). Medium 199 was supplied by Sigma (St. Louis, MI, USA). Milli-Q plus water (Millipore, Bedford, MA, USA) was used for all preparations. TDx kit was purchased from Abbot Laboratories (Abbot Park, IL, USA) for the quantitation of cyclosporin in blood.

II. *Preparation of CS for Administration*

CS was a gift from Dr. Hung-Lung Hou (Chiayi, Taiwan) and identified by macroscopic and microscopic examination. It was ground to fine powder and well suspended in distilled water to 200 mg/mL.

III. *Animals and Drug Administration*

Male Sprague-Dawley rats (300-400 g) were fasted for 12 hr before drug administration. Cyclosporin (2.5 mg/kg) was orally given to rats with and without an oral dose of CS (2.0 g/kg) concomitantly just prior to cyclosporin (n = 6) and 1 hr before cyclosporin (n = 7), respectively, in crossover designs. Both cyclosporin and CS were given via gastric gavage. One week was allowed for washout.

In separate study, cyclosporin was intravenously given to 8 rats (0.8 mg/kg) with and without a concomitant oral dose of CS (2.0 g/kg) in crossover design. CS was given via gastric gavage immediately after the intravenous bolus of cyclosporin via tail vein. One week was allowed for washout. The animal study adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" (published by the Chinese Society for the Animal Science, Taiwan, ROC).

IV. *Blood Collection*

Blood samples (0.3 mL) were withdrawn via cardiopuncture at 20, 40, 60, 180, 300 and 540 min after oral administration and at 5, 10, 20, 40, 60, 180, 300 and 540 min after intravenous bolus. The blood samples were collected in vacutainer tubes containing 8.55 mg of EDTA (Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed within 24 hr.

V. *Determination of Cyclosporin Concentration in Blood*[19,20]

Blood sample (150 μL) was treated with 50 μL of Solubilization Reagent and 300 μL of Whole Blood Precipitation Reagent. After well mix, the mixture was centrifuged at 9,860×g for 5 min to obtain supernatant. Cyclosporin concentration was then measured by a specific monoclonal fluorescence polarization immunoassay. Validation of the calibration curve was carried out by testing three controls with concentrations of 150.0, 400.0 and 800.0 ng/mL right before sample assay. The assay was calibrated for concentrations from 25.0 to 1500.0 ng/mL. The LLOQ (lower limit of quantification) of cyclosporin was 25.0 ng/mL.

VI. *Everted Intestine Sac Study*[21]

Nine Sprague-Dawley rats were fasted for 24 hr and then sacrificed with ether. Segments of jejunum and ileum, each with 25 cm in length, were removed. The segments were washed with iced saline and then everted and ligated at both ends. Subsequently, this sac was incubated for 20 min in 50 mL of Medium 199 solution containing 0.5 g of cordyceps, which was gassed with 95% O_2/5% CO_2 at 37˚C. Afterwards, the sac was filled with 3.0 mL of Medium 199 solution containing 20.0 μg/mL rhodamine 123. Solutions outside the sac (800 μL each) were taken every 20 min up to 100 min as sample. The concentration of rhodamine 123 in each sample was determined fluorometrically using a Luminescence Spectrometer LS-50B (Perkin Elmer, USA).

VII. *Data Analysis*

The area under the serum concentration - time curves ($AUC_{0\text{-}540}$) of oral and intravenous cyclosporin were calculated using noncompartment models of WINNONLIN (version 1.1, SCI software, Statistical Consulting, Inc., Apex, NC, USA), respectively. The peak serum concentration (C_{max}) and the time to peak concentration (T_{max}) were observed from experimental data. Paired and unpaired Student's *t*-test were used for statistical comparisons of *in vivo* and *in vitro* studies, respectively, taking $p < 0.05$ as significant.

RESULTS

Figures 1 and 2 depict the blood profiles of cyclosporin after oral administration of cyclosporin and coadministration with CS concomitantly and 1 hr before

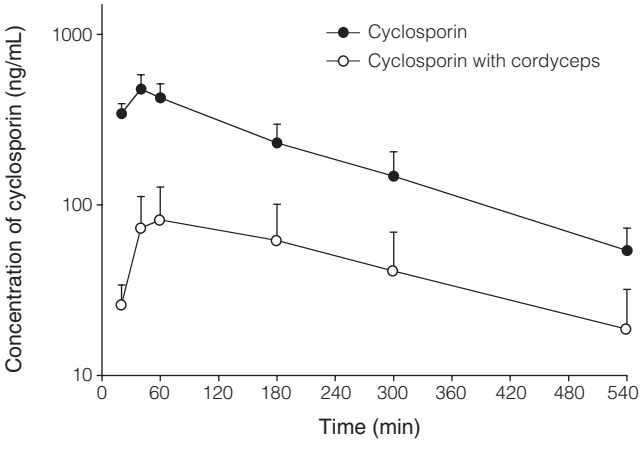

Figure 1. Mean (± S.E.) blood concentration - time profiles of cyclosporin after oral administration of cyclosporin (2.5 mg/kg) (●) and coadministration with 2.0 g/kg cordyceps (○) in 6 rats.

cyclosporin. The pharmacokinetic parameters of cyclosporin for various treatments are given in Tables 1 and 2. The results showed that coadministration of CS concomitantly and 1 hr before cyclosporin significantly decreased C_{max} of cyclosporin by 85.5% and 85.9%, as well as reduced $AUC_{0\text{-}540}$ by 83.2% and 84.4%, respectively. Concomitant administration of CS significantly delayed the T_{max} by 58.3%, whereas coadministration of CS 1 hr before cyclosporin did not affect T_{max}.

Figure 3 depicts the blood profiles of cyclosporin after intravenous bolus of cyclosporin and coadministration with CS. The result showed that the two profiles were largely superposable and the pharmacokinetic parameters were not significantly different between the two treatments.

The results of everted intestine sac study are shown in Figure 4. CS did not significantly alter the efflux transport of rhodamine 123 from serosal to mucosal side for both jejunum and ileum.

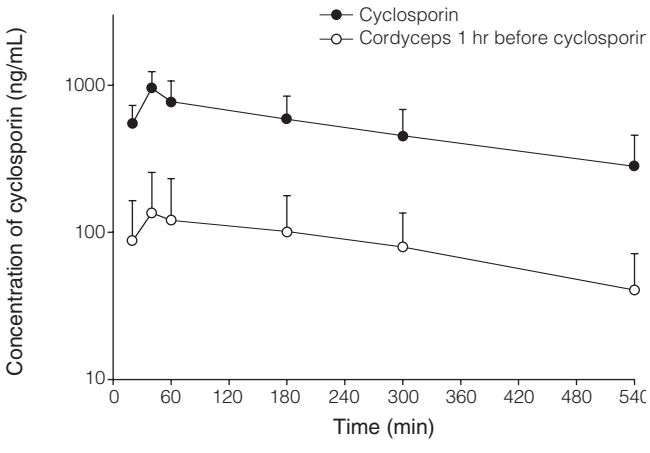

Figure 2. Mean (± S.E.) blood concentration - time profiles of cyclosporin after oral administration of cyclosporin (2.5 mg/kg) (●) and coadministration with 2.0 g/kg cordyceps 1 hr before cyclosporin (○) in 7 rats.

Table 1. Pharmacokinetic parameters of cyclosporin in rats after giving oral cyclosporin (CsA, 2.5 mg/kg) and coadministration with cordyceps (CS, 2.0 g/kg) (n = 6)

Parameters	CsA	CsA + CS	Difference (%)
C_{max}[a] (ng/mL)	491.0 ± 100.7	85.9 ± 44.1	-85.5 ± 5.0[e]
T_{max}[b] (min)	36.7 ± 6.2	50.0 ± 6.8	58.3 ± 35.2[e]
$t_{1/2}$[c] (min)	155.7 ± 29.9	194.8 ± 111.7	20.2 ± 21.9[e]
$AUC_{0\text{-}540}$[d] (μg•min/mL)	106.9 ± 29.0	24.4 ± 15.5	-83.2 ± 8.4[e]

Data expressed as Mean ± S.E.
[a]Concentration of peak blood level.
[b]Time of peak blood level.
[c]Half life.
[d]Area under serum concentration - time curve to the last point.
[e]$p < 0.01$ compared with CsA.

Table 2. Pharmacokinetic parameters of cyclosporin in rats after giving oral cyclosporin (CsA, 2.5 mg/kg) and predosing with cordyceps 1 hr before cyclosporin (CS 1hr, 2.0 g/kg) (n = 7)

Parameters	CsA	CsA + CS 1hr	Difference (%)
C_{max} (ng/mL)	952.9 ± 107.8	140.5 ± 44.4	-85.9 ± 4.1[a]
T_{max} (min)	40.0 ± 0.0	57.1 ± 20.7	42.9 ± 51.7
$t_{1/2}$ (min)	324.5 ± 98.3	293.0 ± 101.5	-5.3 ± 14.1
$AUC_{0\text{-}540}$ (μg•min/mL)	270.2 ± 44.8	44.2 ± 12.8	-84.4 ± 3.7[b]

Data expressed as Mean ± S.E.
[a]$p < 0.001$ compared with CsA.
[b]$p < 0.01$ compared with CsA.

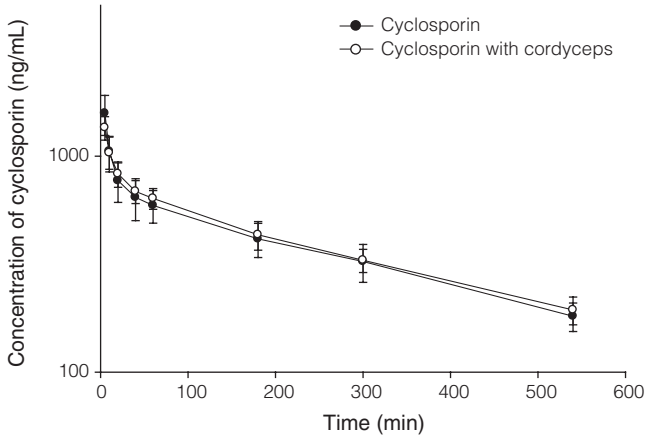

Figure 3. Mean (± S.E.) blood concentration - time profiles of cyclosporin after intravenous bolus of cyclosporin (0.8 mg/kg) (●) and coadministration with 2.0 g/kg (○) cordyceps in 8 rats.

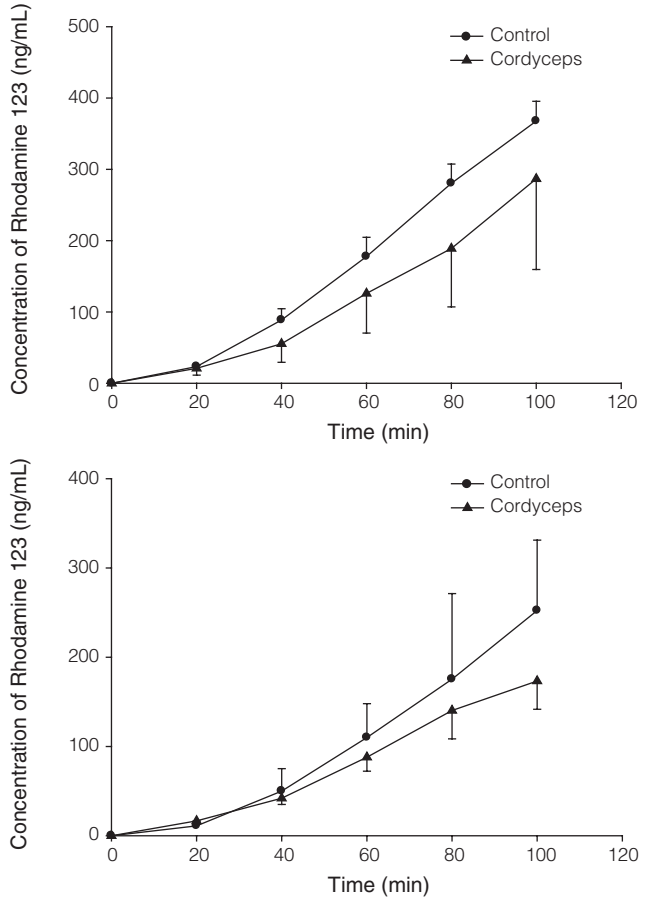

Figure 4. Mean (± S.E.) transport of rhodamine 123 from serosal to mucosal surfaces across the everted jejunum (upper) and ileum (lower) in the absence (control) (●) or presence of 0.5 g/beaker of CS (▲) (n = 3).

DISCUSSION

Due to the variation of cyclosporin absorption among individuals, crossover design was conducted in this study to overcome the great inter-subject difference. This rat model for herb - cyclosporin interaction had been verified by a study of coadministration with St. John's wort and cyclosporin[22], demonstrating a result in good agreement with clinical findings[23]. In this study, the results indicated that coadministration of CS markedly reduced the C_{max} and $AUC_{0\text{-}540}$ of oral cyclosporin, whereas the pharmacokinetics of intravenous cyclosporin were not significantly affected. Apparently, CS markedly decreased the systemic exposure of oral cyclosporin, but conferred no impact on the distribution and elimination of intravenous cyclosporin. Therefore, it can be proposed that CS did not significantly affect the disposition of cyclosporin and thus the interaction should occur during the absorption phase in gastrointestinal tract[24]. When CS was given 1 hr before cyclosporin dosing, the C_{max} and $AUC_{0\text{-}540}$ of cyclosporin significantly decreased in no lesser extent than when CS was coadministered concomitantly, suggesting that direct pharmaceutical interaction between CS and cyclosporin could be excluded.

Cyclosporin is a substrate of Pgp, an efflux transporter[25] which is present in intestine and plays as biochemical barrier of drug absorption[26]. The results of everted gut sac study showed that CS did not alter the function of intestinal Pgp, suggesting that the reduced cyclosporin bioavailability caused by CS cannot be explained by the modulation on intestinal Pgp. Since complex mechanisms of drug absorption were gradually recognized in recent decades, the interaction mechanism between CS and cyclosporin might involve metabolizing enzymes such as CYP3A4 or other ATP-binding cassette (ABC) transporters beside Pgp.

A previous study reported that CS extract inhibited the proliferation and IL-2 production of phytohemagglutinin-stimulated peripheral blood mononuclear cell[13]. Pharmacodynamically, CS and cyclosporin may cause synergistic effect on immunosupression. However, CS coadministration dramatically inhibited the oral bioavailability of cyclosporin and resulted in reduced blood level. Therefore, optimizing the dosage regimen of cyclosporin requires not only the blood levels of cyclosporin, but also the pharmacodynamic interaction between CS and cyclosporin.

In conclusion, cordyceps significantly reduced cyclosporin bioavailability. Concurrent use of cordyceps with cyclosporin is better avoided to ensure the efficacy and safety of cyclosporin.

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