Activation of In Vivo Kupffer Cell Function by Oral Administration of *Cordyceps sinensis* in Rats

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ABSTRACT—We investigated the effect of water extracts of *Cordyceps sinensis* (WECS) on Kupffer cell function in rats. Rats were received a single i.v. injection of a colloidal carbon solution and then the clearance rate from the blood were measured. The rats had been daily administered with WECS, p.o. at a dose of 200 mg/kg for 25 days until the day before the injection of colloidal carbon. The half-life of the colloidal carbon in the blood of rats administered WECS 200 mg/kg was significantly shorter than that of the control rats. This suggests that accelerated function of Kupffer cells is partially involved in the anti-metastatic action of WECS.

Keywords: Water extract of *Cordyceps sinensis* (WECS), Kupffer cell function, Colloidal carbon

Cordyceps sinensis is a fungus parasitic on the larvae of *Lepidoptera* and has been used as one of the most valued traditional Chinese medicines for a long time. Not only the preclinical studies but also the clinical studies on the fermentation product of *Cordyceps sinensis* are conducted in China. *Cordyceps sinensis* has received approval by the National New Drug Review and Approval Committee of the Chinese Ministry of Public Health and has been used in clinics throughout China for the indications of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthenia after severe illness, respiratory diseases, renal dysfunction and renal failure, arrhythmias and other heart diseases, and liver diseases (1). However, the reported effects of *Cordyceps sinensis* on immunological function differ greatly, being in the opposite extremes. It has been reported as an immunosuppressant by some investigators (2-4) and an immunostimulant by others (5-7). We have previously elucidated that water extracts of the cultural fruit body of *Cordyceps sinensis* (WECS) possess anti-metastatic activities using spontaneous metastatic model mice (8). Kupffer cells, resident hepatic macrophages, are the largest populations of the mononuclear phagocyte system in the body and function as a scavenger of foreign or unnecessary substances for the body such as microorganisms, degenerated cells and tumor cells. They exhibit cytotoxicity to tumor cells by means of releasing cytokines (9), arrest circulating tumor cells and control their metastatic growth in the liver (10). In the present study, we examined the effects of WECS on the in vivo clearance of colloidal carbon in the blood of rats to explore the mechanism of anti-metastatic activity of WECS.

The dried fruit bodies of the cultured *Cordyceps sinensis* were produced by Xinhui Xinhan Artificial Cordyceps Factory (Guangdong, China) and supplied by Gunsei Co., Ltd. (Tokyo). They were extracted with hot water (70℃) for 5 min and the extract was then filtered and lyophilized. The lyophilized powder (WECS) was sealed in bottles and kept in a refrigerator (4℃) until use. Specific pathogen-free male Sprague-Dawley rats, 6-week-old (171.2±1.6 g, n=12) for WECS 2 days treatment; 4-week-old (83.6±1.1 g, n=18) for WECS 25 days treatment; and 7-week-old (220.5±2.2 g, n=9) for zymosan treatment were purchased from Japan SLC, Inc. (Hamamatsu). The administration of WECS was started after acclimation of the animals for one week. The rats were maintained in an air-conditioned room (23±2℃ temp. and 60±10% humidity) under an artificial 12-hr light/dark cycle (7:00 a.m.-7:00 p.m.). Diet and water were given ad libitum during the experimental period. We faithfully followed the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. WECS was dissolved in water and administered p.o. daily in a dose of 200 mg/kg body wt./day for 2 days in the WECS 2 days treatment experiment and in a dose of 100 and 200 mg/kg

body wt./day for 25 days in the WECS 25 days treatment experiment until the day before the injection of colloidal carbon. Zymosan A from *Saccharomyces cerevisiae* purchased from Sigma (St. Louis, MO, USA) was dispersed in saline and administered i.v. daily in a dose of 25 mg/kg body wt./day for 2 days until the day before the injection of colloidal carbon according to Bailie et al. (11). The activity of Kupffer cells was determined by a modified method of Sauer et al. (12). On the day after the final WECS or zymosan administration, 8-week-old rats were each anesthetized with pentobarbital sodium salt (65 mg/kg body wt., i.p.) and a control sample of blood (0.1 ml) was removed from the jugular vein using a heparinized syringe with a 23-gauge needle. A colloidal carbon solution, india ink (BACTIDROP), purchased from Remel (Lenexa, KS, USA), was diluted with an equal volume of 1.8% NaCl solution and administered via the tail vein (2 ml of diluted india ink solution/kg body wt.). Blood samples (0.1 ml each) were taken at an interval of 5 min from the jugular vein using heparinized syringes with 23-gauge needles for a total of 30 min. Following collection, 50 μl of blood was placed in 1 ml of a 0.1% solution of $NaHCO_3$ to lyse the red blood cells, and this solution was analyzed spectrophotometrically at 640 nm. The log of the absorbance of the blood lysate was plotted vs. time and each half-life of the colloidal carbon in the blood was calculated. After the final sampling of the blood, the liver and spleen were excised and weighed. Relative liver and spleen wt. (%) were calculated by the following equation:

(liver or spleen wt. (g) / body wt. (g)) $\times$ 100.

The data are expressed as the mean$\pm$S.E.M. of 4–6 animals. Statistical analyses were performed by ANOVA followed by Fisher's PLSD (protected least significant difference) test or Student's *t*-test using the Stat View software package (Abacus Concepts, Inc., Calabasas, CA, USA). A difference was considered significant when $P<0.05$.

On the first day of WECS administration, the body wt. of control, WECS 100 mg/kg administered and 200 mg/kg administered rats were 108.9$\pm$1.0, 109.5$\pm$1.8 and 108.6$\pm$2.2 g, respectively, in the WECS 25 days treatment experiment. On the day of the colloidal carbon injection, no significant difference in the body wt. among the three groups of rats was also observed. Similarly, WECS 2 days treatment had no effect on body wt. gain. We also measured wet wt. of the spleen and liver in rats after the clearance experiments of colloidal carbon. The liver and spleen wt. and the relative liver and spleen wt. of the WECS administered rats were not different from those of control rats in both WECS 2 and 25 days treatment (Table 1). These findings indicate that orally administered WECS was quite safe because it has no toxic effect on the body wt. gain of rats. Manabe et al. have reported similar data that a water extract of *Cordyceps sinensis* has no effect on wt. gain, liver wt. or relative liver wt. after 3-week oral administration (200 mg/kg) to mice. Moreover, no pathological change is observed in the liver specimens from *Cordyceps sinensis* extract-treated mice (13).

Cowper et al. have demonstrated that colloidal carbon is taken up exclusively by non-parenchymal cells and predominantly by Kupffer cells based on the data from light-microscopy, cell-separation and histological studies (14). In accordance with their report, we evaluated the phagocytic function of Kupper cells using colloidal carbon. The colloidal carbon clearance value ($t_{1/2}$) in WECS 200 mg/kg administered rats was significantly shorter by 36% than that of control rats and 35% shorter than that of WECS 100 mg/kg administered rats in the WECS 25 days treatment experiment (Fig. 1a). That is to say, WECS 200 mg/kg administration had an activating effect, but 100 mg/kg administration had no effect on the Kupffer cell phagocytic function. In contrast, the effect of WECS 200 mg/kg administration for 2 days on the rate of colloidal carbon clearance in rats was not significant.

Table 1. No effect of WECS on body wt., liver wt., spleen wt., relative liver wt. and relative spleen wt. in rats

Groups	Body wt. (g)	Liver wt. (g)	Spleen wt. (g)	Relative liver wt. (%)	Relative spleen wt. (%)
WECS 2 days treatment					
Control	230.9±4.7	7.07±0.19	0.577±0.018	3.06±0.08	0.250±0.008
WECS (200 mg/kg, p.o.)	236.5±3.5	7.22±0.20	0.640±0.026	3.05±0.07	0.271±0.010
WECS 25 days treatment					
Control	221.3±8.4	7.07±0.31	0.559±0.044	3.19±0.03	0.252±0.016
WECS (100 mg/kg, p.o.)	239.7±3.4	8.01±0.14	0.626±0.031	3.34±0.05	0.261±0.012
WECS (200 mg/kg, p.o.)	229.3±9.0	7.42±0.46	0.592±0.045	3.22±0.07	0.258±0.016

Data are expressed as the mean±S.E.M. of 6 rats.

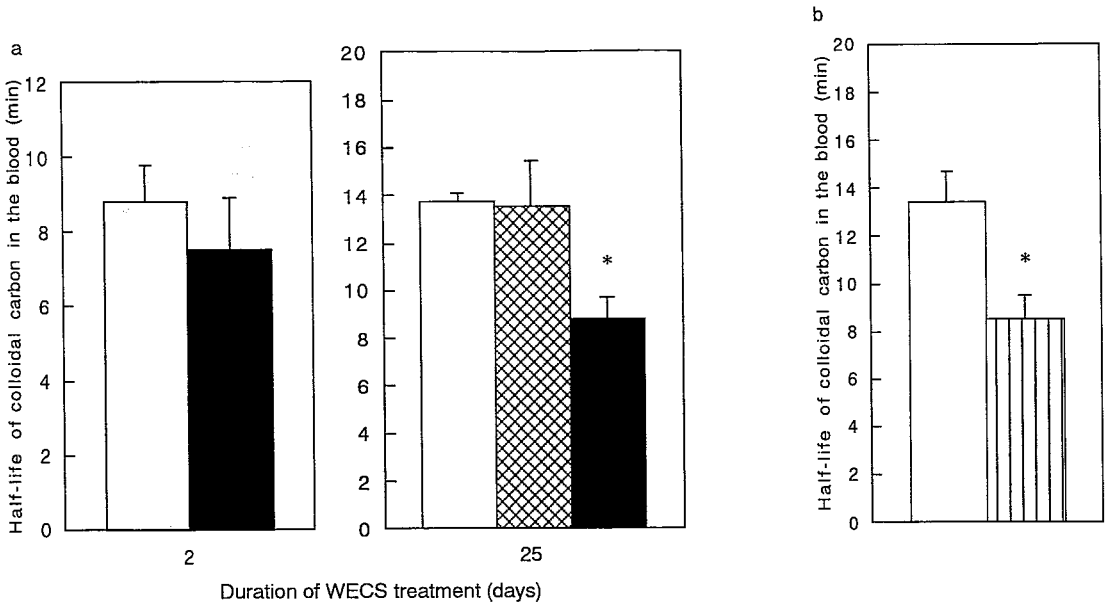

Fig. 1. Effects of WECS and zymosan on the in vivo clearance of colloidal carbon. a: WECS (200 mg/kg body wt. (■), 2 days treatment or 100 (▨) and 200 (■) mg/kg body wt, 25 days treatment) were daily administered p.o. to rats for 2 and 25 days until the day before injection of colloidal carbon. b: Zymosan (25 mg/kg body wt. ▥) was daily administered i.v. to rats for 2 days until the day before injection of colloidal carbon. Rats were evaluated for their ability to clear colloidal carbon from the blood. Data are expressed as means±S.E.M. of 4–6 animals. *Significantly different from the respective control (□) ($P<0.05$).

As a comparative study, we used zymosan, an agent that increases hepatic macrophage numbers (11). The colloidal carbon clearance value ($t_{1/2}$) in zymosan-administered rats was significantly shorter by 37% than that of control rats (Fig. 1b). The effect of intravenously administered zymosan (25 mg/kg body wt. for 2 days) was similar to that of orally administered WECS (200 mg/kg body wt. for 25 days) under our experimental condition. In some cases, the activation of Kupffer cell function is harmful to the body. For instance, Kupffer cell function is critical to cadmium-induced hepatocellular necrosis (12). Nevertheless, Kupffer cells function as the first-line defense against hematogenous liver metastasis via their phagocytosis and cytotoxicity (10). Liu et al. have reported that *Cordyceps sinensis* increases interleukin-1 and interferon production by cultured rat Kupffer cells (15). Their report endorses our experimental results. Our findings are the first evidence that WECS accelerate the activity of in vivo Kupffer cell phagocytosis in rats. The anti-metastatic effect of WECS is shown in mice (8). However, we could not use mice for the Kupffer cell study because of their insufficient blood volume. This activity in rats might be partially involved in the anti-metastatic action of WECS in mice. Chinese investigators have reported that oligosaccharides and polysaccharides isolated from natural *Cordyceps sinensis* stimulate macrophage function (1). According to their results, these are highly probable candidates for effective components in WECS to stimulate Kupffer cell function. Nevertheless, we suspect that there are some effective components other than oligosaccharides and polysaccharides because WECS were administered to rats orally in our experiment. It is necessary to elucidate the specific component in WECS that activates the Kupffer cell in the future.

In conclusion, we elucidated that orally administered WECS accelerate the activities of Kupffer cell function without weight loss and toxicity in rats. According to our experimental results, WECS may be clinically useful as an immune activator to prevent tumor metastasis.

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