Inhibitory Effect of Cordycepin on Experimental Hepatic Metastasis of B16-F0 Mouse Melanoma Cells

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Abstract. In a previous study performed by our group, we demonstrated that the water extract of Cordyceps sinensis (WECS) significantly prevented tumor metastasis from the spleen to the liver, using B16-F0 mouse melanoma cells as a model. In this study, we investigated the anti-metastatic activity of cordycepin (3’-deoxyadenosine), one of the components of WECS, using an identical model of mice injected with B16-F0 cells into the spleen. All mice inoculated with B16-F0 cells died due to liver metastases via the portal vein from the spleen. Control mice not administered cordycepin exhibited higher serum levels of alanine aminotransferase (ALT) due to damage to the liver by metastasized B16-F0 cells from the spleen, and survival times ranged from 17 to 22 days after tumor inoculation. Cordycepin was intraperitoneally administered to mice, and resulted in significantly lower serum ALT levels and longer survival times than those observed in control mice. Taken together, these results indicate that cordycepin may be the active ingredient in C. sinensis exerting an anti-metastatic effect, and may be a potential candidate anti-metastatic agent.

Cordyceps sinensis, a fungus parasitized on the larva of Lepidoptera, has been valued as a traditional medication and tonic food in China for over 300 years. Because the natural products of C. sinensis are so rare and difficult to obtain in uniform composition, cultured products have been developed. A mycelial fermentation product of C. sinensis, was approved by the National New Drug Review and Approval Committee of the Chinese Ministry of Public Health, and has been used in clinics for fatigue, night sweats, male and female hyposexuality, impotence, hyperglycemia, hyperlipidemia, asthenia after severe illness, respiratory diseases, renal dysfunction and renal failure, arrhythmias and other heart, and liver diseases (1).

In previous studies, we focused on the cultural fruiting body of C. sinensis instead of the mycelium and investigated the effects of a water extract of C. sinensis (WECS) on an experimental hepatic metastatic melanoma model in mice. Furthermore, we attempted to elucidate the mechanisms for the anti-metastatic activity of WECS by focusing on the invasion step, which is one of the key steps in the tumor cell metastatic process. As a result, WECS was shown to have anti-metastatic activity by inhibiting hepatocyte growth factor (HGF)-accelerated tumor invasiveness of mouse melanoma cells (2). We determined that WECS exhibits anti-invasive activity, in part, by increasing tissue inhibitor of metalloproteinase-1 (TIMP-1) secretion by melanoma cells (3).

In the present study, we examined the effects of cordycepin (3’-deoxyadenosine), one of the components of WECS, on an experimental model of hepatic metastatic melanoma in mice to elucidate the pharmacologically-active component in WECS that is responsible for its anti-metastatic activity.

Materials and Methods

Materials. Cordycepin (Figure 1) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). Dulbecco’s modified Eagle’s medium (DMEM) with L-glutamine was from Invitrogen Co. (Grand Island, NY, USA). Dulbecco’s phosphate-buffered saline without calcium and magnesium [DPBS (−)] was from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). EDTA trypsin solution (EDTA, 0.02%); trypsin, 0.1% and penicillin/streptomycin solution (penicillin, 50,000 U/ml; streptomycin, 50 mg/ml) were obtained from Cosmo Bio Co., Ltd. (Tokyo, Japan).
Animals. Regarding syngeneic animals, specific pathogen-free male C57BL/6Cr mice (seven weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and used in experiments after one-week acclimation. Mice were maintained in an air-conditioned room (23±2°C and 60±10% humidity) under an artificial 12-hour light/dark cycle (7:00 a.m.-7:00 p.m.). Food and water were given ad libitum during the experimental period. All procedures followed the Guiding Principles for the Care and Use of Laboratory Animals approved by the Animal Experimentation Committee in Mukogawa Women’s University (study approval #080037).

Cells. The mouse epithelial-like melanoma cell line, B16-F0, was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells passaged fewer than 33 times were used in all experiments and cultured in DMEM containing 10% FBS and a 0.1% penicillin/streptomycin solution.

Assay of experimental hepatic metastasis of melanoma cells in mice. Sub-confluent cells were harvested with EDTA trypsin solution and resuspended at appropriate densities in DPBS (–). The left lower back of mice was minimally cut and the spleen was exposed under anesthesia with pentobarbital. B16-F0 cells (1×10^5/50 μl) were injected via the spleen into syngeneic C57BL/6Cr mice.

Cordycepin was dissolved in DPBS (–) and administered intraperitoneally to mice daily at a dose of 0 (control), 0.5, or 5.0 mg/kg for 19 days after tumor inoculation. The spleen was removed seven days after tumor cell inoculation under anesthesia with pentobarbital to avoid the influence of the primary focus. Cordycepin was administered intraperitoneally daily at a dose of 0, 0.5, or 5.0 mg/kg for 19 days after tumor inoculation. Blood was removed from the tail vein 14 days after tumor inoculation and ALT levels in the serum were measured. Data are expressed as the mean±S.E.M. of 6 or 7 mice. *p<0.05 vs. Cordycepin 0 mg/kg.

Statistical analyses. Statistical analyses were performed by ANOVA followed by Fisher’s protected least significant difference test using the Stat View software package (SAS Institute, Cary, NC, USA). Kaplan-Meier survival analysis was used to calculate survival curves, followed by the log-rank test to determine significance using PRISM Version 4 (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered significant at p<0.05.

Results

Effect of cordycepin on AST and ALT serum levels in experimental hepatic metastatic model mice. Both AST and ALT levels were unchanged among control mice (cordycepin 0 mg/kg), and mice administered cordycepin at 0.5 and 5.0 mg/kg at 10 days after tumor inoculation (data not shown). Serum ALT levels were unchanged in controls and mice administered 0.5 mg/kg cordycepin, while levels in mice administered 5.0 mg/kg were significantly lower than those of control mice at 14 days after tumor inoculation (p<0.05, Figure 2).

Effect of cordycepin on survival in an experimental model of hepatic metastatic melanoma using B16-F0 cells. All mice inoculated with B16-F0 cells died due to liver metastasis via the portal vein from the spleen. Control mice died between days 17 and 22, with the average period of survival being

Figure 1. Chemical structure of cordycepin.

Figure 2. Effect of cordycepin on serum alanine aminotransferase (ALT) levels in liver metastatic melanoma-bearing mice. Sub-confluent B16-F0 cells (1×10^5) were injected via the spleen into syngeneic C57BL/6Cr mice. The spleen was removed after seven days to avoid the influence of the primary focus. Cordycepin was administered intraperitoneally daily at a dose of 0, 0.5, or 5.0 mg/kg for 19 days after tumor inoculation. Blood was removed from the tail vein 14 days after tumor inoculation and ALT levels in the serum were measured. Data are expressed as the mean±S.E.M. of 6 or 7 mice. *p<0.05 vs. Cordycepin 0 mg/kg.
19.6±0.5 days after B16-F0 cell inoculation. The average survival durations of mice administered cordycepin at 0.5 and 5.0 mg/kg were 21.7±0.8 and 22.9±1.3 days, respectively. Significant differences were recorded between control and groups administered cordycepin at 0.5 and 5.0 mg/kg (p<0.05). However, there was no difference between the two doses used in this experiment (Figure 3).

Discussion

In a previous study we demonstrated that WECS exhibits anti-metastatic activity using the same liver metastatic model as the one used in this study (2). Next, we attempted to elucidate the effective components in WECS since WECS is a crude powder that contains many bioactive ingredients, including nucleosides (adenosine, guanosine, and uridine) (4), cordycepin (5), water-soluble polysaccharides (6), and peptides (7). We investigated cordycepin as a potential candidate responsible for the antimetastatic activity of WECS and administered it to model mice using the same schedule for WECS in this experiment since cordycepin is a pure component isolated from WECS that is commercially available. Furthermore, using a high-performance liquid chromatography-electrochemical detection (HPLC-ECD) system, we determined the content of cordycepin in WECS to be 2.34% w/w, which was much higher than that of adenosine (0.12% w/w in WECS) (8).

As a result, we confirmed that cordycepin significantly prolongs the survival of experimental hepatic metastatic model mice. In the previous study, we indicated the antimetastatic effect of WECS using the same liver metastatic model as employed in this study. As a result, the same WECS treatment (50 mg/kg for twenty days) significantly reduced the number of liver metastatic nodules and significantly prolonged the survival of mice in two experiments (2). Therefore, we solely conducted the survival experiment in this study. We also demonstrated that serum ALT levels in cordycepin-administered mice were significantly lower than those in control mice.

Mouriaux et al. examined the relevance of liver function tests (LFTs), including AST, ALT, gamma glutamyltransferase, lactate dehydrogenase, and alkaline phosphatase, for the early detection of liver metastasis of uveal melanoma. They showed that the positive predictive value (PPV) of all tests ranged from 9.4% to 38.6%, while the negative predictive value (NPV) was 90% or greater. They concluded that LFTs were not helpful for detecting early metastasis. However, the high NPV suggests that LFT screening can allow clinicians to reassure the patient when LFT results are negative (9). We agree with their conclusion especially regarding ALT.
Chen et al. reported that cordycepin, as one of the two major compounds from the ethanolic extracts of fruiting bodies of *A. cinnamomea*, significantly inhibited matrix metalloproteinase-9 (MMP-9) and MMP-2 expression in culture media of CL1-0 human lung adenocarcinoma cells (10). On the other hand, we previously reported that cordycepin significantly increased tissue inhibitor of metalloproteinase-1 (TIMP-1) secretion from B16-F0 cells, similar to the levels obtained with the WECS treatment (3). Both Chen et al.’s and our findings suggest that cordycepin possesses antimetastatic activity by reducing the invasiveness of tumor cells through either inhibiting MMPs or accelerating TIMP-1.

In conclusion, our results indicate that cordycepin may be a potential candidate anti-metastatic agent.

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**References**


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