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Current perspective

Anticancer and antimetastatic effects of cordycepin, an active component of *Cordyceps sinensis*

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ABSTRACT

Cordyceps sinensis, a fungus that parasitizes on the larva of Lepidoptera, has been used as a valued traditional Chinese medicine. We investigated the effects of water extracts of *Cordyceps sinensis* (WECS), and particularly focused on its anticancer and antimetastatic actions. Based on *in vitro* studies, we report that WECS showed an anticancer action, and this action was antagonized by an adenosine A_3 receptor antagonist. Moreover, this anticancer action of WECS was promoted by an adenosine deaminase inhibitor. These results suggest that one of the components of WECS with an anticancer action might be an adenosine or its derivatives. Therefore, we focused on cordycepin (3′-deoxyadenosine) as one of the active ingredients of WECS. According to our experiments, cordycepin showed an anticancer effect through the stimulation of adenosine A_3 receptor, followed by glycogen synthase kinase (GSK)-3β activation and cyclin D_1 suppression. Cordycepin also showed an antimetastatic action through inhibiting platelet aggregation induced by cancer cells and suppressing the invasiveness of cancer cells via inhibiting the activity of matrix metalloproteinase (MMP)-2 and MMP-9, and accelerating the secretion of tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 from cancer cells. In conclusion, cordycepin, an active component of WECS, might be a candidate anticancer and antimetastatic agent.

1. Introduction

Cordyceps sinensis is a fungus that parasitizes on larvae of Lepidoptera and has been used as a herbal tonic in traditional Chinese medicine for over 300 years. Many papers have reported the diverse pharmacological activities of *C. sinensis* (1,2). Natural products of *C. sinensis* are too rare to obtain and very expensive. In addition, the content of each component of natural products is variable and it might be difficult to check their quality. Therefore, we chose the cultured fruiting body of *C. sinensis* produced by Xinhui Xinhan Artificial Cordyceps Factory (Guangdong, China) and supplied by Gunsei Co., Ltd. (Tokyo, Japan) as our experimental material, and investigated the pharmacological effects of hot water extracts (70 °C for 5 min) of *C. sinensis* (WECS). We investigated the action of WECS on cancer, particularly on metastasis. As active ingredients of WECS, we focused on cordycepin (3′-deoxyadenosine) and examined its anticancer and antimetastatic effects and the mechanisms of these effects. It has been reported that cordycepin interacts in biochemical processes, including nucleic acid synthesis, platelet aggregation, metastasis, inflammatory reactions, apoptosis, and cell cycle signaling (3). In this review, we mainly present our research findings on cordycepin, as an active ingredient of WECS.

2. Direct cytotoxicity of WECS against cancer cells via stimulation of adenosine A_3 receptor

In *in vitro* studies, Nakamura et al. investigated the anticancer effect of WECS against B16 mouse melanoma (B16) and Lewis lung carcinoma (LLC) cells, and WECS showed direct cytotoxicity against both B16 and LLC cells at 10 and 30 μg/mL (4). Nakamura et al. indicated that WECS (100 μg/mL) induced the apoptosis of B16-F10 mouse melanoma cells after 48-h exposure *in vitro*, as determined by both the TdT-mediated dUTP-biotin nick end labeling (TUNEL) method and the detection of a DNA ladder (5). Lee et al. also demonstrated that cordycepin induced apoptosis in human

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prostate PC-3 cells through a mitochondria-mediated, caspase-dependent pathway (6). Yoshikawa et al. reported that WECS (10 μg/mL) markedly inhibited the growth of B16-BL6 mouse melanoma (B16-BL6) cells, LLC cells, HT1080 human fibrosarcoma (HT1080) cells, and CW-2 human colon carcinoma (CW-2) cells, and the inhibitory effect of WECS was significantly antagonized by 1 μM 3-ethyl 5-benzyl 2-methyl-6-phenyl-4-phenylethynyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate (MRS1191), a selective adenosine A_3 receptor antagonist. Furthermore, WECS included 2.34% w/w cordycepin and 0.12% w/w adenosine as components according to the HPLC-electrochemical detection (ECD) system (7). That is, one of the active ingredients of WECS inhibited the proliferation of four cancer cell lines by the stimulation of adenosine A_3 receptors, and this active ingredient may be cordycepin and not adenosine.

3. Cordycepin, an active ingredient of WECS, and its direct cytotoxicity against cancer cells

In *in vitro* studies, Nakamura et al. demonstrated that cordycepin showed marked inhibitory effects on the growth curves of B16-BL6 cells (IC_{50} = 39 μM) and LLC cells (IC_{50} = 48 μM), while adenosine and 2′-deoxyadenosine (up to 100 μM) had no effect on the growth of the two cancer cell lines. Among the adenosine receptor agonists and antagonists used (up to 100 μM), only 2-chloro-N6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide (Cl-IB-MECA), a selective adenosine A_3 receptor agonist, notably inhibited the growth of B16-BL6 cells (IC_{50} = 5 μM) and LLC cells (IC_{50} = 14 μM). Furthermore, the cancer growth inhibitory effect of cordycepin was antagonized by MRS1191 (8). Thus, cordycepin exerts direct cytotoxicity against mouse melanoma and lung carcinoma cells by stimulating adenosine A_3 receptors. These results also support cordycepin as a potent active ingredient of WECS.

4. Promotion of anticancer effects of WECS and cordycepin by 2′-deoxycoformycin (DCF), an adenosine deaminase inhibitor

In *in vitro* studies, Yoshikawa et al. attempted to elucidate the combined effect of DCF, an adenosine deaminase inhibitor, with WECS and cordycepin on the growth curves of B16-BL6 and LLC cells. As a result, the anticancer effect of WECS on the growth curves of the two cancer cell lines increased over three-fold in combination with DCF. In addition, DCF significantly promoted the anticancer effect of cordycepin by approximately three hundred-fold (9). Consequently, DCF is a potent adjuvant for WECS. In other words, one of the effective components of WECS is metabolized by adenosine deaminase. These phenomena indicate that cordycepin may be one of the active components of WECS.

5. Cordycepin exerts an anticancer effect through the stimulation of adenosine A_3 receptor followed by glycogen synthase kinase-3β (GSK-3β) activation and cyclin D_1 suppression

In *in vitro* studies by Yoshikawa et al., a radioligand binding assay using [^{125}I]-AB-MECA, a selective adenosine A_3 receptor agonist, revealed that B16-BL6 cells express adenosine A_3 receptors and that cordycepin binds to these receptors. Yoshikawa et al. also confirmed the involvement of adenosine A_3 receptors in the action of cordycepin using MRS1523 and MRS1220, specific adenosine A_3 receptor antagonists. Next, indirubin, a GSK-3β inhibitor, antagonized the growth suppression of B16-BL6 cells induced by cordycepin. Furthermore, the level of cyclin D_1 protein in B16-BL6 cells was decreased by cordycepin based on Western blot analysis (10). Taken together, cordycepin inhibits the proliferation of mouse melanoma cells by stimulating adenosine A_3 receptors followed by the Wnt signaling pathway, including GSK-3β activation and cyclin D_1 inhibition. Ko et al. demonstrated that cordycepin enhanced proteasome-dependent degradation and inhibited the nuclear translocation of β-catenin in U937 human leukemic monocyte lymphoma (U937) cells. Furthermore, cordycepin-reduced β-catenin stability was restored by the addition of a GSK-3β inhibitor (SB216763), indicating that this stability is mediated by the activation of GSK-3β (11). Their results strongly support our findings.

6. Combined effects of WECS (p.o.) and methotrexate (MTX) on hematogenous lung metastasis in mice

In *in vivo* studies, combined treatment with WECS and MTX of C57BL/6J mice intravenously inoculated with B16-BL6 cells was conducted. WECS (200 and 500 mg/kg) in drinking water was given to mice from one week before to 20 days after cancer inoculation (for 27 days). MTX was administered s.c. daily to the mice at a dose of 15 mg/10 mL/kg for 20 days from the date of cancer inoculation. Although MTX caused a significant and severe decrease in the body weight compared with that in control mice starting 16 days after the start of administration, the mice given both MTX and WECS did not show a significant decrease in body weight. The survival time of mice given the combination of MTX and WECS (200 mg/kg) was significantly longer than that of the control mice (5). WECS might be beneficial for the prevention of cancer metastasis as an adjuvant agent in cancer chemotherapy, and it also reduces the adverse effects of chemotherapeutic agents.

7. Inhibitory effect of WECS (i.p.) on hepatic metastasis of cancer cells in mice

In *in vivo* studies, Kubo et al. investigated the antimetastatic activity of WECS using a mouse model injected with B16-F0 mouse melanoma (B16-F0) cells into the spleen. WECS (50 mg/kg/day for 20 days after cancer inoculation) administered intraperitoneally significantly reduced the number of metastatic surface nodules of B16-F0 cells in the liver of C57BL/6Cr mice, and significantly prolonged their survival. Furthermore, they examined the effect of WECS on the hepatocyte growth factor (HGF)-accelerated invasion of B16-F0 cells using a chemo-invasion assay *in vitro*. WECS (1 μg/mL) was shown to significantly reduce HGF-accelerated B16-F0 cell invasion (12). Moreover, Kubo et al. investigated the effect of WECS on tissue inhibitor of metalloproteinase (TIMP)-1 secretion from B16-F0 cells in order to identify clues to the mechanism underlying the anti-invasive action of WECS. As a result, WECS (1 μg/mL) significantly increased the secretion of TIMP-1 from B16-F0 cells (13). These results suggest that WECS has an antimetastatic action through inhibiting the invasiveness of cancer cells by accelerating the secretion of TIMP-1 from cells.

8. Anticancer activity of cordycepin (p.o.) in mice

In *in vivo* studies, the anticancer effect of orally administered cordycepin was examined in C57BL/6Cr mice inoculated with B16-BL6 cells. B16-BL6 (1×10^6) cells were inoculated subcutaneously into the right footpad of mice. At two weeks after the cell inoculation, the enlarged primary cancer lump was weighed. Cordycepin (15 mg/kg per day), administered orally to the mice for two weeks from the date of cancer inoculation, significantly reduced the wet weight of the primary cancer by 36% compared to that of the untreated control mice, without any loss of body weight or systemic toxicity (14). These results show that orally administered cordycepin inhibits melanoma cell growth in mice with no side effects.

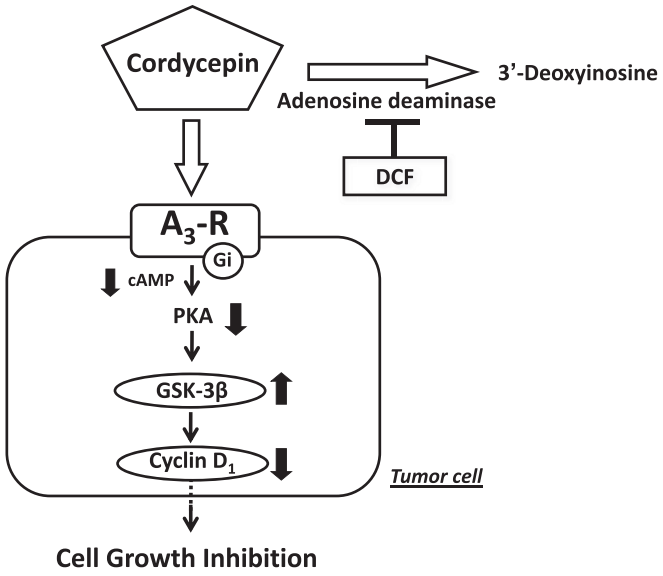

Fig. 1. Scheme showing the mechanism of the anticancer effect of cordycepin and its degradation by adenosine deaminase. A_3-R: adenosine A_3 receptor (Gi protein coupled receptor), PKA: cAMP-dependent protein kinase, GSK-3β: glycogen synthase kinase-3β, DCF: 2′-deoxycoformycin.

9. Inhibitory effect of cordycepin (i.p.) on hepatic metastasis of cancer cells in mice

In *in vivo* studies, Sato et al. investigated the anti-metastatic activity of cordycepin using a mouse model injected with B16-F0 cells into the spleen. Cordycepin was administered intraperitoneally daily at a dose of 0, 0.5, or 5.0 mg/kg for 19 days after cancer inoculation. All C57BL/6Cr mice inoculated with B16-F0 cells died due to liver metastasis via the portal vein from the spleen. Cordycepin at 0.5 and 5.0 mg/kg resulted in significantly longer survival times than those observed in control mice (15). Kubo et al. investigated the effect of cordycepin on TIMP-1 secretion from B16-F0 cells in order to identify clues to the mechanism underlying the anti-invasive action of cordycepin. Cordycepin was shown to significantly accelerate the release of TIMP-1 from cells (13). Jeong et al. also reported that cordycepin inhibited the expression and activity of MMP-2 and MMP-9 and simultaneously increased levels of TIMP-1 and TIMP-2 using LNCaP human prostate carcinoma cells (16). Taken together, cordycepin may be a potential candidate antimetastatic agent through inhibiting the activity of MMPs and accelerating the release of TIMPs from cancer cells.

10. Inhibitory effect of cordycepin (i.v.) on hematogenous metastasis of cancer cells accelerated by adenosine-5′-diphosphate (ADP)

In *in vivo* studies, Yoshikawa et al. investigated whether platelet aggregation accelerates hematogenous metastasis of B16-F1 mouse melanoma (B16-F1) cells in C57BL/6Cr mice and the effect of cordycepin on hematogenous metastasis accelerated by ADP. ADP significantly increased the number of metastatic lung nodules in mice injected intravenously with B16-F1 cells in a dose-dependent manner, and cordycepin significantly reduced the number of metastatic nodules of B16-F1 cells formed in the lung accelerated by ADP injected simultaneously with B16-F1 cells (17). Accordingly, ADP accelerated hematogenous metastasis and cordycepin had an inhibitory action on hematogenous metastasis of B16-F1 cells via the blocking of ADP-induced platelet aggregation *in vivo*.

11. Activation of Kupffer cell function by WECS (p.o.) in rats

In *in vivo* studies, Sprague-Dawley rats received a single i.v. injection of a colloidal carbon solution, and then the clearance rate from the blood was measured. The rats had been administered WECS p.o. daily at a dose of 200 mg/kg for twenty-five days until the day before the injection of colloidal carbon. The half-life of the colloidal carbon in the blood of rats administered WECS at 200 mg/kg was significantly shorter than that of the control rats (18). These results indicate that orally administered WECS activates one of the immune systems in rats.

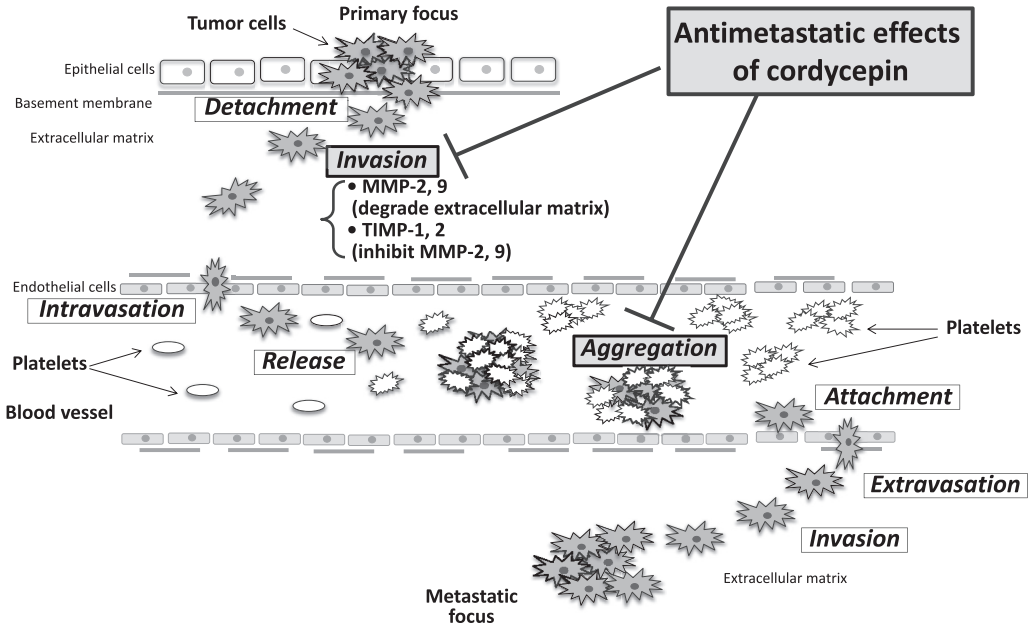

Fig. 2. Scheme showing the mechanism of the antimetastatic effect of cordycepin. MMP: matrix metalloproteinase, TIMP: tissue inhibitor of metalloproteinase.

12. Beneficial effects of WECS and cordycepin on atherosclerosis *in vitro* and *in vivo* (p.o.)

In *in vitro* studies, Yamaguchi et al. indicated that WECS exhibited potent antioxidant and antilipid peroxidation activities and inhibited the accumulation of cholesteryl ester in macrophages via the suppression of low-density lipoprotein (LDL) oxidation (19). Furthermore, Yamaguchi et al. showed that WECS administered orally prevented cholesterol deposition in the aorta of atherosclerotic ICR mice by the inhibition of LDL oxidation mediated by free radicals rather than by reduction of the serum lipid level (20). Guo et al. reported that cordycepin administered i.g. at 25 and 50 mg/kg for two weeks prevented hyperlipidemia in Syrian golden hamsters fed a high-fat diet via the activation of AMP-activated protein kinase (AMPK) (21). In addition, Won et al. demonstrated that cordycepin injected orally at 10 mg/kg for 14 days attenuated neointimal formation by inhibiting reactive oxygen species-mediated responses in vascular smooth muscle cells in Sprague-Dawley rats (22). Accordingly, cordycepin, as an active ingredient of WECS, may exert beneficial effects on the formation of atherosclerotic lesions induced by oxidative stress.

13. Conclusion

According to our data, cordycepin might be one of the active ingredients of WECS since the anticancer effect of WECS was antagonized by MRS1191, MRS1523, and MRS1220, specific adenosine A_3 receptor antagonists, and enhanced by the addition of DCF, an adenosine deaminase inhibitor. We revealed that cordycepin exhibited an anticancer action through the stimulation of adenosine A_3 receptor followed by GSK-3β activation and cyclin D_1 suppression (Fig. 1). Cordycepin also showed an antimetastatic action through the inhibition of platelet aggregation initiated by ADP released from cancer cells and reduction of the invasiveness of cancer cells via inhibiting the activity of MMP-2 and MMP-9 and accelerating the secretion of TIMP-1 and TIMP-2 from those cells (Fig. 2). Cordycepin, an active component of WECS, is expected to be a candidate anticancer and antimetastatic agent.

Conflicts of interest

The authors declare no conflict of interest.

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