Int.J.Curr.Microbiol.App.Sci (2018) 7(2): 988-997

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 7 Number 02 (2018)
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.122

Specific Antiproliferative Activity against Several Human Cancer Cells Possessed by *Cordyceps militaris* Grown in Viable Pupa of Silkworm Raised under Sterile Environment

Yukiko Ogawa[1*], Hiroko Murakami[2], Takuya Kuraoka[3], Ryukichi Mine[2] and Hidemitsu Kobayashi[3]

[1]*Divisions of Infection Control and Prevention, Department of Pharmacy, Faculty of Pharmaceutical Science, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan*
[2]*Minejumokuen Co., Ltd., Nonoshima, Goshi, Kumamoto, 861-1103, Japan*
[3]*Divisions of Microbiology, Department of Pharmacy, Faculty of Pharmaceutical Science, Nagasaki International University, 2825-7 Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan*

**Corresponding author*

Keywords

Cordyceps militaris, Silkworm, *Bombyx mori*, Anticancer activity, Medicinal plants

Article Info

Accepted:
10 January 2018
Available Online:
10 February 2018

A B S T R A C T

We succeeded in large volume artificial cultivation of *Cordyceps militaris*, using silkworm live pupa obtained by aseptic breeding. The appearance (shape, color, and size) of fruiting bodies of cultured bacteria was almost the same as that of wild one. We investigated the antiproliferative inhibitory effect on eleven human cancer cells *in vitro* using the water extracted fraction obtained from this fruiting body homogenate. As a result, this soluble fraction effectively inhibited the growth of some cancer cells in a concentration-dependent manner. In particular, the effect on leukemia U937 cells was remarkable, but there was almost no effect on bile duct adenocarcinoma TYBDC-1 cells or pancreatic KP-3L cancer cells. In addition, almost no effect was exerted on three normal cells (human hepatocyte (NHH), human mammary epithelial cells (NHME), human epidermal melanocytes (NHEM)) at the same concentration. Therefore, it was revealed that the human cell proliferation inhibitory effect of the *C. militaris* water extract fraction greatly differs in susceptibility depending on the type of cancer cell under the concentration range not affecting normal cells. Furthermore, the anti-cancer effect of *C. militaris* was associated with an induction of cell cycle arrest and caspase-mediated apoptosis. This fact suggests that some compounds contained in metabolic fluids of these mushrooms can be lead compounds leading to the development of anticancer drugs with very few side effects.

Introduction

Ophiocordyceps sinensis (formerly known as *Cordyceps sinensis*) originating from China is considered effective in maintaining and enhancing health, and is a well-known raw material in widely-distributed herbal medicine (Bok *et al.,* 1999; Nakamura *et al.,* 1999; Paterson *et al.,* 2008). However, since commercially available *O. sinensis* produced in China is collected from wild

specimens, the distribution volume is extremely low and cost is very high. For the common person, this valuable product is beyond his reach.

On the other hand, *C. millitaris* produced in Japan has been reported as not only having health enhancing activity, but also a strong anticancer effect, and has especially attracted attention (Jeong *et al.,* 2012; Song *et al.,* 2016). Regarding components in *C. militaris*, the chemical compounds ergosterol and cordycepin are considered to possess anti-tumorigenic activity (Nallathamby *et al.,* 2015; Shao *et al.,* 2016). Ergosterol has been reported to inhibit colon adenocarcinoma HT29 cell growth at a concentration which hardly influences fibroblast growth (Kobori *et al.,* 2007), while cordycepin is considered to be a specific metabolite of *C. militaris* (Jeong *et al.,* 2013; Mollah *et al.,* 2012). As a result of a survey on 24 species of genus *Cordyceps* including *O. sinensis*, only *C. militaris* was reported to contain cordycepin (Hur H., 2008; Kang *et al.,* 2017; Yue *et al.,* 2013).

Since cordycepin is a nucleic acid metabolic substance, it functions to inhibit DNA and RNA biosynthesis, and is thought to induce positive cell death in abnormal cells (malignant tumors). In clinical reports after 1961, cordycepin has been consistently shown to inhibit cancer cells such as Ehrlich ascite tumors, melanoma, lung cancer, acute lymphatic leukemia (Chen *et al.,* 2013; Jeong *et al.,* 2012; Kodama *et al.,* 2000; Lee *et al.,* 2013; Lee *et al.,* 2016; Shao *et al.,* 2016; Tian *et al.,* 2015; Yoshikawa *et al.,* 2004). Regarding the use cordycepin as a new drug for acute leukemia and HIV, clinical experiments in the final certification stage are still underway (De *et al.,* 2015; Müller *et al.,* 1991; Wang *et al.,* 2017). There are also reports that cordycepin functions to control growth of bad bacteria in the intestine, but do not influence lactic acid bacteria (Ahn *et al.,* 2000).

One reason why research in genus *Cordyceps* has not progressed up to now is that the fruiting body is small and very difficult to detect, and collecting wild strains from the natural environment is extremely difficult. Moreover, artificial cultivation has not been established for most species. Even if conditions for artificial cultivation were discovered, another reason of difficulty is the extremely slow growth rate, with 1-2 years required for the stationary regions to become research worthy. Furthermore, in general artificial cultivation without the use insects, achieving the same amount of active components as wild strain is questionable.

We carried out artificial cultivation of *C. militaris* by producing a large quantity of silkworms in a sterilized room, and using them as a live culture medium. Components from the obtained fruiting bodies were extracted with water (CM), and anti-growth inhibition activity for various types of human cancer cells was considered.

Materials and Methods

Strains

The stroma of *Cordyceps* sp. grown on the pupae of Lepidoptera moth (unidentified) were collected in the summer of 1996 at Siping Jilin, China. The fungus was identified as *C. militaris* by microscopic examination of the stroma and ascospore. The isolate was obtained from the ascospore. The fungus was cultured on a slant culture medium containing peptone (10 g/l), yeast extract (10 g/l), glucose (30 g/1) and agar (1.5%) at 25°C for 10 days. When the conidia matured, they were taken out of the tube and suspended in sterilized water. The concentration of conidial suspension was adjusted to 10^7 conidia/ml. This suspension was used in the inoculation experiments.

Rearing of host silkworm

The silkworm, *Bombyx mori*, was reared in a sterilized indoor environment on an artificial diet. The eggs were disinfected with a 3% formaldehyde for 15 min and then hatched in sterilized equipment.

The larvae were kept at 29°C from the first to third instar, and at 25°C from the fourth instar till maturation. After pupation, the pupae to be used as hosts were removed from the cocoons. Some 5th instar larvae of day 2 were also used as larval hosts.

Artificial inoculation

The artificial inoculation was performed the method of Chen *et al.,* (Chen *et al.,* 2002). Three methods were used to inoculate the larvae and pupae with the conidial suspension; (1) spraying method in which 3 ml of the conidial suspension per 20 insects was sprayed onto the surface of the host silkworm once a day, for two days; (2) dipping method in which the whole insect body was dipped in the conidial suspension for 20 sec, once a day, for two days; and (3) hypodermic injection in which 0.1 ml of the conidial suspension was directly injected into the silkworm body with a syringe. The inoculated larvae and pupae were reared at 25°C and 90% RH, in darkness, and at 23-25 °C and 85-90% RH, respectively. Also, twenty larvae and pupae were used for each treatment. Furthermore, to compare the infection rate at different injection sites, the conidial suspension was injected into the head, thorax and abdomen of the male and female pupae. One hundred pupae were used for each injection. When the infected larvae and pupae became sclerosed with *C. militaris*, they were placed in a culture glass jar (commercial mayonnaise jar, 65 mm in diam, 130 mm in height) at 20°C and 90% RH, and at a photoperiod of 12L-12D (200-300 lux) to develop the stromata

Chemicals

Cell Counting Kit-8 including 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophhenyl)-2H-tetrazolium, monosodium salt (WST-8) was from Dojindo Co. (Kumamoto, Japan). RPMI 1640, E-MEM, L-15 and Ham's F12 medium were from Nissui Pharmaceutical Co. (Tokyo, Japan). Fetal bovine serum (FBS) was from Life Technologies Co. (Carlsbad, CA, USA). Penicillin- streptomycin was from Roche Diagnostics K. K. (Tokyo, Japan). Trypan blue solution was from Nacalai Tesque, Inc. (Kyoto, Japan).

Cells

Human hepatoma HepG2 cells and breast cancer MCF-7 cells were obtained from Institute of Development, Ageing and Cancer, Tohoku University. Human promonocytic leukemia U937 cells, human colon adenocarcinoma COLO201 cells, human bile duct cancer TYBDC-1 cells, human gastric carcinoma KATO III cells, human lung squamous carcinoma HARA cells, human pancreatic tumor KP-3L cells, and human malignant melanoma GAK cells were obtained from the JCRB Cell Bank. Human colon adenocarcinoma Caco-2 cells and human breast cancer MDA-MB-231(MDA) cells were purchased from DS Pharma Biomedical Co. (Tokyo, Japan). Normal human hepatocytes (NHH), normal human mammary epithelial cells (NHME), Normal human epidermal melanocytes (NHEM) were purchased from Promo Cell Co. (Heidelberg, Germany). U937, COLO201, HARA and KP-3L cells were maintained in RPMI 1640 medium. Caco-2, MCF-7 and HepG2 cells were grown E-MEM. MDA cells were grown L-15 medium, and TYBDC-1 cells were maintained DMEM and Ham's F12 medium. KATOIII cells were grown IMDM, and GAK cells were maintained Ham's F12 medium. All cell cultures were supplemented with heat-inactivated fetal bovine serum 10% (v/v), penicillin (100 IU/ml), and streptomycin (100 □ g/ml) at 37 °C in an atmosphere of 95% air/ 5% CO_2.

Cell viability and cytotoxic assay

Cells (2×10^4, in 90 □ l solution) were cultured in 96-well flat-bottom plate and treated with various concentrations of CM for 48 h at 37 °C in an atmosphere of 95% air and 5% CO_2. Cytotoxic activity and cell viability and cell growth were evaluated by trypan blue (0.5% (w/v)) exclusion and by the WST-8 assay (10 □ l), respectively. The reduction in proportion of living cells was assayed by measurement of absorbance at 450 nm (reference, 600 nm) using the GloMax Multi Detection System.

DNA fragmentation analysis

U937 and MDA cells (1×10^6 cells/dish) were plated in 6-cm dish and then treated with or without CM. After the treatments, the cells were washed with ice-cold PBS and resuspended in lysis buffer (50 mM Tris-HCl, pH8.0, 10 mM EDTA, and 0.5 % SDS) with 0.2 mg/ml RNase A for 30 min at 50 °C. Proteinase K was added and cells were incubated overnight. The DNA was separated using a 1.8 % agarose gel and visualized under UV illumination after staining with ethidium bromide.

Analysis of FITC-labeled annexin V binding and propidium iodide incorporation

Cell apoptosis was detected using Annexin V-FITC Apoptosis detection kit I (BD Biosciences, San Diego, CA, USA) according to the manufacturer's instructions. U937 cells ($1x10^6$ cells/dish) were plated in 6-cm dish, and treated with or without CM. After treatment with CM for 6, 12 and 24 h, the cells were centrifuged and washed twice with cold PBS. The cells were re-suspended in binding buffer and then incubated with Annexin V-FITC and PI for 15 min at room temperature in the dark. Samples were then analyzed by FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA). The cells ($2x10^4$ cells) were counted for each sample.

Caspase activation assay

Cells (5×10^5) were cultured in the presence of CM for 24 h at 37°C. The activation of caspase-3 was evaluated by the addition of hydrolysed artificial substrates Caspase-3 colorimetric protease assay kit (Bio Vision, Milpitas, CA).

Statistical analysis

The results of experiments are presented as mean ± standard error (SE). Differences in means were evaluated by two-tailed Student's *t*-test with *P* values <0.05 considered to be statistically significant.

Results and Discussion

Artificial cultivation using live sterilized silk worms

Harada *et al.,* and Chen *et al.,* first reported a cultivation method (Harada *et al.,* 1995; Chen *et al.,* 2002; Sato *et al.,* 2002) for *C. militaris* using parasitic insects as the culture media. At this time, we allowed a type of genus *Cordyceps*, *C. militaris*, to effectively form on sterilized silkworms, and established a cultivation system to grow a large amount of fruiting bodies. Figure 1A shows the sericulture system in a sterilized room, Figure 1B shows the propagation of sterilized silkworms. Fourteen days after the inoculation, the primordia of stroma, colored orange, emerged from the dead pupae (Fig. 1C). Lastly, approximately 60 days after the inoculation, the stromata matured and grew longer than 7 cm in length (Fig. 1D). The fruiting body had similar form, size, and color compared to wild *C. militaris.* Moreover, the amount of active components per 100g of each of these fruiting bodies was 180 mg cordycepin and 603 mg ergosterol. These were approx. 36 times and 6 times the amount in *C. militaris* produced in Taiwan and which is widely distributed. In addition, these components were not detected in silkworms which were not inoculated.

Anti-proliferative activity of *C. militaris* on human cancer cells

We screened the growth inhibition activity of *C. militaris*, which was successfully and artificially cultivated from sterilized silk worms, on various types of human cancer cells. Among 11 kinds of cancer cells, almost no effect was observed for KP-3L, and the effect on Caco-2 and TYBDC-1 was also weak. On the other hand, effect on U937, Colo201, MDA-MB-231, and KATOIII was strong, and extremely strong growth inhibition activity was especially seen for leukemia U937 cells (Table 1 and Fig. 2). At this concentration, there was hardly any effect on normal cells (human hepatocytes (NHH), human mammary epithelial cells (NHME), and human epidermal melanocytes (NHEM)) (Table 1 and Fig. 3).

Whether the difference in cell specificity by these active components depends on components in the mulberry leaves which are fed to sterilized silkworms is still under consideration. However, the fact that *C. militaris* grown from sterilized silkworms acted differently according to the type of cancer cell at a concentration that does not affect normal cells, the bioactive forms contained the these mushrooms may differ depending on the compounds obtained from artificial cultivation.

Confirmation of apoptosis induction by CM

To determine whether the inhibition of cell proliferation by CM was due to apoptosis, DNA fragmentation was investigated. DNA fragmentation was detected using agarose gel electrophoresis of cells treated with RNase and proteinase K. CM caused DNA fragmentation that was characteristic of apoptosis in U937 cells (Fig. 4A).

Table.1 Growth inhibitory effect of silkworm pupa-cultivated *Cordyceps militaris*

Cancer cell line	1.25	0.63
U937	+++	+++
Colo201	+++	++
Caco-2	+	−
MCF7	++	+
MDA-MB-231	+++	++
HepG2	++	++
TYBDC-1	+	−
KATOⅢ	+++	++
HARA	++	++
KP-3L	−	−
GAK	++	++

Normal cell line	1.25	0.63
NHH	−	−
NHME	−	−
NHEM	−	−

Cytotoxic activity of silkworm pupa-cultivated *Cordyceps militaris* (CM). Cells were treated with CM (1.25 and 0.63 mg/ml) for 48 h . Then cell viability was determined by WST-8 assay and trypan blue dye exclusion assay. Cell viability; +++ : 30% or less, ++ : 31-50%, + : 51-65% and - : more than 66%. Data are the means of triplicate assay mean ± SE.

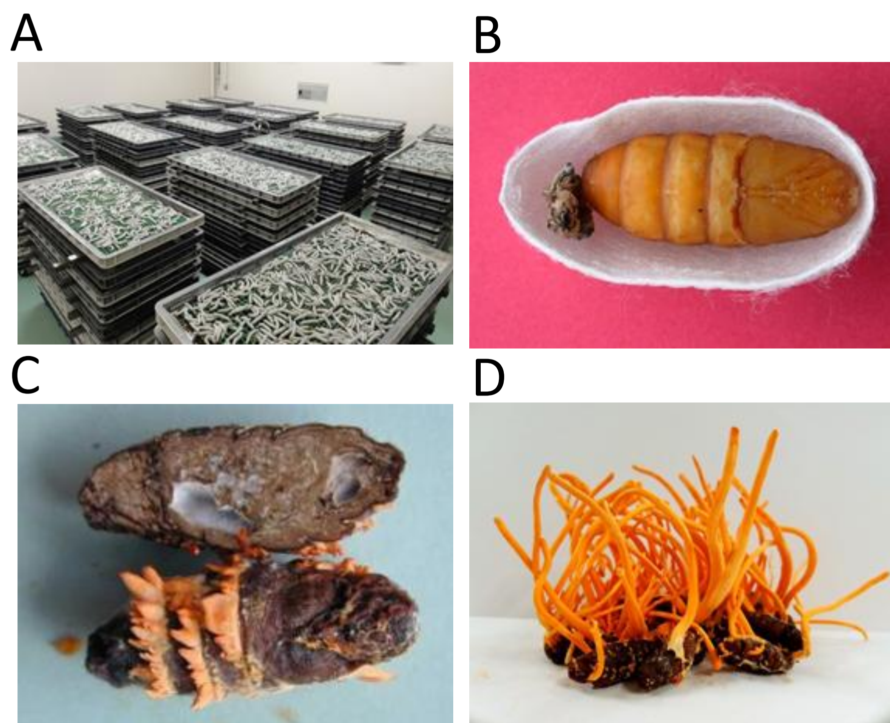
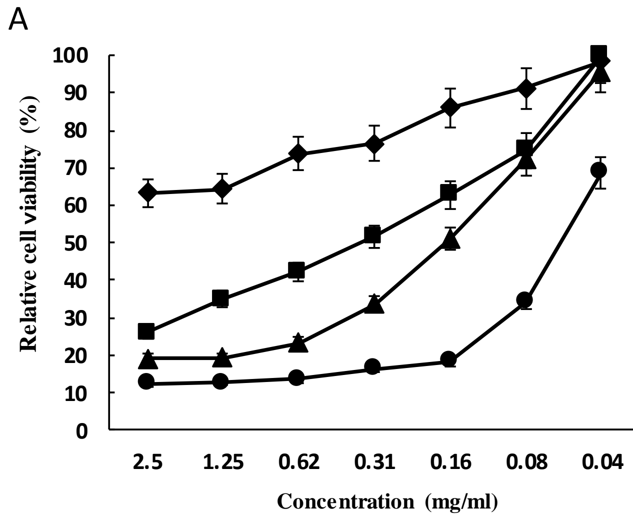


Fig. 1 Pupae of *Bombyx mori* infected with *Cordyceps militaris* (CM).
(A) Sericulture system in a sterilized room.
(B) Propagation of sterilized silkworms. Pupa before infection.
(C) Primordia of stroma colored orange emerged from the silkworm pupae.
(D) Stromata of *C.militaris* grew on the pupae.



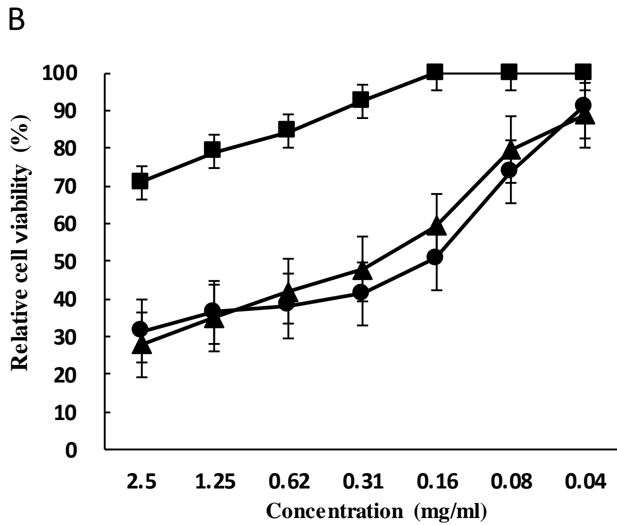


Fig. 2 Antiproliferative effect of CM on human cancer cells
Cells were treated with CM (2.5-0.04 mg/ml) for 48 h. Then cell viability was determined by WST-8 assay and trypan blue dye exclusion assay. Error bars: SE from three different cell preparations assayed individually. (A) U937 (●), MDA-MB-231 (▲), HepG2 (■) and TYBDC (♦). (B) HARA(●), GAK(▲) and KP-3L(■).

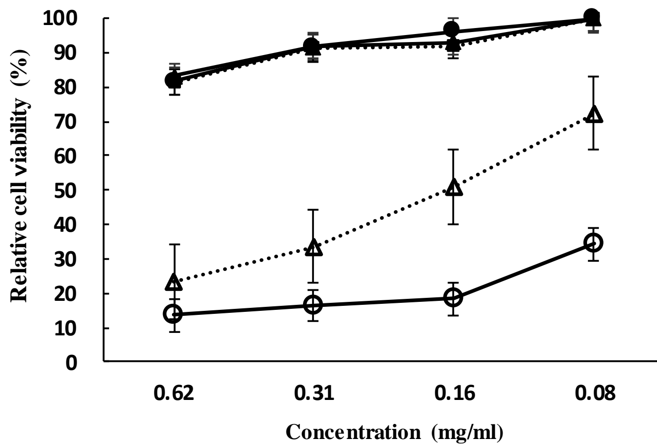


Fig. 3 Effect of CM on the Growth of human normal cells
Cells were treated with CM (2.5-0.08 mg/ml) for 48 h . Then cell viability was determined by WST-8 assay and trypan blue dye exclusion assay. Error bars: SE from three different cell preparations assayed individually. U937 (○), MDA-MB-231 (△), NHH (●), NHME (▲) and NHEM (■).

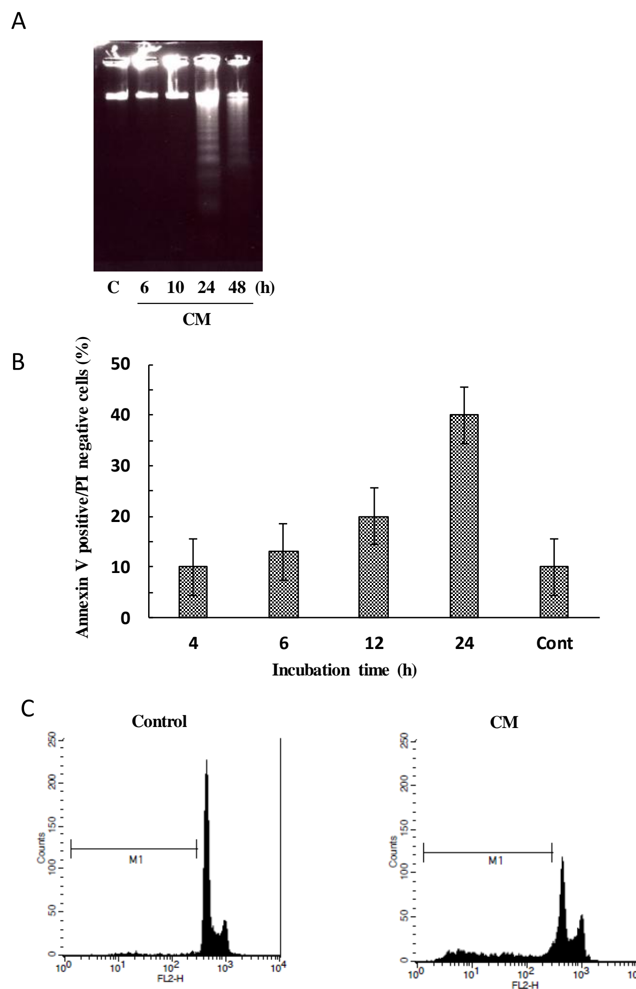


Fig. 4 Effect of CM on apoptosis induction.
(A) Induction of DNA fragmentation by CM. U937 cells were treated with CM (1 mg/ml) for the times indicated. DNA fragmentation was analyzed by agarose gel electrophoresis. (B) Analysis of FITC-labeled annexin V binding and propidium iodide incorporation. U937 cells were treated with CM (1 mg/ml) for the times indicated, and analyzed by flow cytometry after staining with FITC-annexin V and PI. The data shown are representative of three independent experiments with similar results. Error bars: SE from three different cell preparations assayed individually. (C) Increase of the sub-G1 phase cells by CM. U937 cells were treated with CM (1 mg/ml) for 48 h, and analyzed by flow cytometry after staining with PI. The data shown are representative of three independent experiments with similar results.

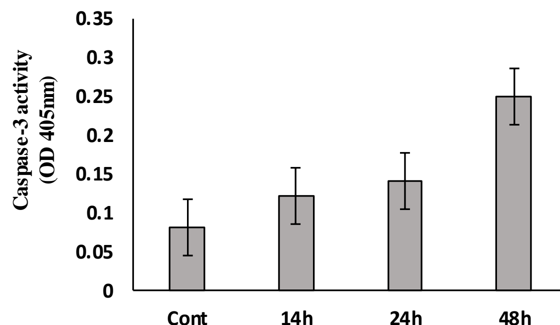


Fig. 5 CM induced apoptosis through a caspase-mediated pathway in U937 cells.
Involvement of the caspase cascade in CM-induced apoptosis. U937 cells were treated with CM (1mg/ml) for the times indicated. Cells were lysed and treated with substrate of caspase-3 and the activity were measured. Error bars: SE from three different cell preparations assayed individually.

CM induced a time-dependent increase in the binding of annexin V to phosphatidylserine (PS) that migrated toward the outer layer of the cell membrane (Fig. 4B). The presence of PS is another biochemical characteristic of apoptosis. Furthermore, a FACS analysis of the sub-G_1-phase population of propidium iodide-stained cells revealed a marked increase in the sub-G_1 population of CM-treated cells (Fig. 4C). These results suggest that CM inhibits the proliferation of leukemia cells by inducing apoptosis.

Analysis of apoptotic mechanisms induced by CM

To determine the mechanism underlying the apoptosis-inducing effect of CM on U937 cells, caspase activation was assessed. U937 cells treated with CM for 48 h activated caspase-3 (Fig. 5).

C. sinensis including genus *Cordyceps* are rare and its metabolites show various bioactivity depending on the parasitizing bacteria and type of insect, and has been distributed throughout the Asia region as an effective folk medicine to prevent or delay the progress of diabetes, metabolic diseases affecting the cardiovascular system, and cancer (Bhatti *et al.,* 2013; Cheng *et al.,* 2013; Ma *et al.,* 2015: Oh *et al.,* 2008; Yu *et al.,* 2016). However, research reports which demonstrate scientific evidence of efficacy are few, due to source scarcity. We have established an insect biological culturing method which applies ancient Japanese sericulture techniques, and could successfully produce a large amount of *C. militaris*, and have begun screening for anticancer efficacy on various cancer cells. While seeking to develop an anticancer agent using genus *Cordyceps*, toxicity to healthy organs, in other words, occurrence of side-effects is of concern. According to our research results, within the concentration range of *C. militaris* which showed efficacy in breast cancer cells, cell growth inhibition of normal human mammary epithelial cells was not observed. In this experiment, effective and specific growth inhibition effect on leukemia cells, breast cancer cells, colon cancer cells and gastric cancer cells, even at a concentration which does not influence normal cells, could be demonstrated.

In particular, an extremely strong growth inhibition effect on leukemia cells and breast cancer cells was observed. This is the first time the production of a large amount and a stable supply of *C. militaris* using sterilized silkworms, and the use of *C. militaris* to screen for various cancer cells, have been reported. According the above information, various components contained in *C. militaris*, including cordycepin, can be expected to become leading compounds which can contribute to the development of anticancer drugs with extremely few side effects.

Apoptosis is a mechanistically driven form of cell death that is either developmentally regulated or activated in response to specific stimuli or various forms of cell injury (Cummings *et al.,* 2016). In cancer biology, it is now evident that many cancer cells circumvent the normal apoptotic mechanisms to prevent their self-destruction. Therefore, it would be advantageous to shift the balance in favor of apoptosis over mitosis in cancer prevention and chemotherapy. A major feature of apoptosis is cleavage of chromosomal DNA into nucleosome units (Larsen *et al.,* 2015). These changes result from the proteolytic cleavage of various intracellular polypeptides, which is most often caused by a family of cysteine-dependent proteases called caspases (Galluzzi *et al.,* 2015; Mitra *et al.,* 2015). In the present study, nuclear fragmentation and increased annexin V binding were observed in leukemia cells treated *C. militaris* indicating the induction of apoptosis. Furthermore treatment with *C. militaris* activated caspase-3. This result suggests that *C. militaris* induces apoptosis through the caspase-dependent pathway.

In order to identify the bioactive forms of *C. militaris*, we are currently working towards isolated purification of the active fractions using high-performance liquid chromatography after fractioning by various organic solvents and determining the chemical structures by focusing on instrumental analysis such as NMR method, and mass spectrometry.

On the other hand, experiments to clarify anticancer activity mechanisms of the active fractions which have been purified by chromatography, and toxicity tests on mice have

carried out accurately, and publication of the details are planned for the next thesis. In the near future, *C. militaris* is expected to become a valuable resource for combined therapy with an anti-cancer drug, at a dosage which does not show side effects, and a leading compound for novel anticancer agents. Stable supply of rare *C. militaris* using sterilized silk worms has become possible, and is considered to be major progress towards the clinical use of new anticancer drugs.

Acknowledgements

This research was supported by a grant from the Small and Medium Enterprise Agency, Ministry of Economy, Trade and Industry.

References

Ahn Y.J., Park S.J., Lee S.G., Shin S.C., Choi D.H., 2000. Cordycepin: selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against Clostridium spp. *J. Agric. Food Chem.*, 48(7), 2744-2748.

Bok, J.W., Lermer, L., Chilton, J., Klingeman, H.G., Towers, G.H., 1999. Antitumor sterols from the mycelia of *Cordyceps sinensis*. *Phytochemistry*, 51, 891-898.

Bhatti, M.T., Freedman, S.M., Mahmoud, T.H., 2013. Fingolimod Therapy and Mocular Hemorrhage. *J. neuroophthalmol*. 33(4), 370-372.

Chen, R., Ichida, M., 2002. Infection of the silkworm, *Bombyx mori*, with *Cordyceps militaris*. *J. Inect Biotech. Sericol.* 71, 61-63.

Chen, Y.H., Wang, J.Y., Pan, B.S., Mu, Y.F., Lai, M.S., So, E.C., Wong, T.S., Huang, B.M., 2013. Cordycepin enhances cisplatin apoptotic effect through caspase/MAPK pathways in human head and neck tumor cells. *Onco. Tagets Ther.* 25, 983-998.

Cheng, Y.W., Chen, Y.I., Tzeng, C.Y., Chang, C.H., Lee, Y.C., Chen, H.C., Tsai, C.C., Hsu, T.H., Lai, Y.K., Chang, S.L., 2013. Aqueous extracts of *Cordyceps militaris* (*Ascomycetes*) lower the levels of plasma glucose by activating the cholinergic nerve in streptozotocin-induced diabetic rats. *Int J Med Mushrooms,* 15, 277-286.

Cummings, R.J., Barbet G., Bongers, G., Hartmann, B.M., Gettler, K., Muniz, L., Furtado, G.C., Cho, J., Lira, S.A., Blander, J.M., 2016. Different tissue phagocytes sample apoptotic cells to direct distinct homeostasis programs. *Nature*, 539(7630), 565-569.

De C.E., 2015. Curious (Old and New) antiviral nucleoside analogues with intriguing therapeutic potential. *Curr. Med. Chem.*, 22(34), 3866-3880.

Galluzzi, L., López-Soto, A., Kumar, S., Kroemer, G., 2016. Caspases Connect Cell-Death Signaling to Organismal Homeostasis. *Immunity*, 44(2), 221-231.

Harada Y., Akiyama N., Yamamoto K., Shirota Y., 1995. Production of *Cordyceps militaris* by fruit bodies on artificially inoculated pupae of *Mamestra brassicae* in the laboratory. *Nippon Kingakukai Kaiho*, 36, 67-72.

Hur H., 2008. Chemical ingredients of *Cordyceps militaris*. *Mycobiology*, 36(4), 233-235.

Larsen, B.D., Sørensen C.S., 2016. The caspase-activated DNase: apoptosis and beyond. *FEBS J*., doi: 10.1111/febs.13970.

Jeong, J.W., Jin, C.Y., Park, C., Han, M.H., Kim, G.Y., Moon, S.K., Kim, C.G., Jeong, Y.K., Kim, W.J., Lee, J.D., Choi, Y.H., 2012. Inhibition of migration and invasion of LNCaP human prostate carcinoma cells by cordycepin through inactivation of Akt. *Int. J.Oncol.* 40, 1697-1704.

Jeong, M.H., Lee, C.M., Lee, S.W., Seo, S.Y., Kang, B.W., Jeong, Y.K., Choi, Y.J., Yang, K.M., Jo, W.S., 2013. Cordycepin-enriched *Cordyceps militaris* induces immunomodulation and tumor growth delay in mouse-derived breast cancer. *Oncol. Rep*., 30, 1996-2002.

Kang N., Lee H.L., Park I., Seo Y.S., 2017. Development of high cordycepin-producing *Cordyceps militaris* strains. *Mycobiology*, 45(1), 31-38.

Kobori, M., Yoshida, M., Ohnishi-Kameyama, M., Shinmoto, H., 2007. Ergosuterool peroxide from an edible mushroom suppresses inflammatory responses in RAW264.7 macrophages and growth of HT29 colon adenocarcinoma cells. *Br. J. Pharmacol.,* 150, 209-219.

Kodama, E.N., McCaffrey, R.P., Yusa, K., 2000. Mitsuya, H. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive (TdT+) leukemic cells. *Biochem Pharmacol.* 59, 273-281.

Lee, H.H., Park, C., Jeong, J.W., Kim, M.J., Seo, M.J., Kang, B.W., Park, J.U., Kim,

G.Y., Choi, B.T., Choi, Y.H., Jeong, Y.K, 2013. Apoptosis induction of human prostate carcinoma cells by cordycepin through reactive oxygen species-mediated mitochondrial death pathway. *J. Oncology,* 42, 1036-1044.

Lee, W.S., Li, H.H., Sheng, Y., Jian D.J., Shao, Y.R., 2016. Cordycepin induces apoptosis in human liver cancer HepG2 cells through extrinsic and

intrinsic signaling pathway. *Oncol.Lett*., 12, 995-1000.

Ma, L., Zhang S., Du, M., 2015, Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice. *Nutr. Res*., 35(5), 431-439.

Mitra, S., Wewers, M.D., Sarkar A., 2015. Mononuclear Phagocyte-Derived Microparticulate Caspase-1 Induces Pulmonary Vascular Endothelial Cell Injury. *PLoS One*, 10(12), e0145607. doi: 10.1371/journal.pone.0145607

Mollah M.L., Park D.K., Park H.J., 2012. *Cordyceps militalis* grown on germinated soybean induces G2/M cell cycle arrest through downregulation of cyclin B1 and cdc25c in human colon cancer HT-29 cells. *Evid Based Complement Alternat. Med*., 2012, doi: 10.1155/2012/249217.

Müller W.E., Weiler B.E., Charubala R., Pfleiderer W., Leseman L., Sobol R.W., Suhadolnik R.J., Schröder H.C., 1991. Cordycepin analogues of 2', 5'-oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. *Biochemistey*, 30(8), 2027-2033.

Nakamura, K., Yamaguchi, Y., Kagota, S., Kwon, Y.M., Shinozuka, K., Kunitomo, M., 1999. Inhibitory Effect of *Cordyceps sinensis* on Spontaneous Liver Metastasis of Lewis Lung carcinoma and B16 Melanoma Cells in Syngenic Mice. *Jpn. J. Pharmacol*. 79, 335-341.

Nallathamby, N., Guan-S, L., Vidyadaran, S., Abd, M.S.N., Raman, J., Sabaratnam, V., 2015, Ergosterol of *Cordyceps militaris* Attenuates LPS Induced Inflammation in BV2 Microglia Cells. *Nat. Prod. Commun*., 10(6), 885-886.

Oh, J.Y., Baek, Y.M., Kim, S.W., Hwang, H. J., Hwang, H. S., Lee, S. H., Yun, J. W., 2008. Apoptosis of human hepatocarcinoma (HepG2) and neuroblastoma (SKN-SH) cells induced by polysaccharides-peptide complex produced by submerged mycelial culture of an entomopathogenic fungus *Cordyceps sphecocephala*. *J. Microbiol. Biotechnol*., 18, 512-519.

Paterson, R.R.M., 2008. *Cordyceps* - A traditional Chinese medicine and another fungal therapeutic biofactory. *Phytochemistry,* 69, 1469-1495.

Sato, H., Shimizu, M., 2002. Storomata production for *Cordyceps militaris* (Clavicipitales: Clavicipitaceae) by infection of hyphal bodies to alternative host insects. *Appl. Entomol. Zool,* 37, 85-92.

Shao, L.W., Huang, L.H., Yan, S., Jin, J.D., Ren, S.Y., 2016. Cordycepin induces apoptosis in human liver cancer HepG2 cells through extrinsic and intrinsic signaling pathways. *Oncol. Lett.*, 12(2), 995-1000.

Song J.J., Wang Y., Teng M., Zhang S., Yin M., Lu J., Liu Y., Lee R., Wang D., Teng L., 2016. *Cordyceps militaris* induces tumor cell death via the caspase-dependent mitochondrial pathway in HepG2 and MCF-7 cells. *Mol. Med. Rep*., 13, 5132-5140.

Tian, X., Li, Y., Shen, Y., Li, Q., Wang, Q., Feng, L., 2015. Apoptosis and inhibition of proliferation of cancer cells induced by cordycepin. *Oncol. Lett*., 10(2), 595-599.

Wang Y., Mo H., Gu J., Chen K., Han Z., Liu Y., 2017. Cordycepin induces apoptosis of human acute monocytic leukemia cells via downregulation of the ERK/Akt signaling pathway. *Exp. Ther. Med*., 14(4), 3067-3073.

Yoshikawa, N., Nakamura, K., Yamaguchi, Y., Kagota, S., Shinozuka, K., Kunimoto, M., 2004. Antitumor activity of cordycepin in mice. *Clin. Exp. Pharmacol. Physiol.* Suppl 2, S51-3.

Yu, S.H., Dubey, N.K., Li, W.S., Liu, M.C., Chiang, H.S., Leu, S.J, Shieh, Y.H., Tsai,.FC., Deng, WP., 2016. *Cordyceps militaris* Treatment Preserves Renal Function in Type 2 Diabetic Nephropathy Mice. *PLoSOne*, 11(11): e0166342. doi:10.1371/journal.pone. 0166342.

Yue K., Ye M., Zhou Z., Sun W., Lin X., 2013. The genus *Cordyceps*:a chemical and pharmacological review. *J. Pharm. Pharmacol*., 65(4), 474-493.

How to cite this article:

Yukiko Ogawa, Hiroko Murakami, Takuya Kuraoka, Ryukichi Mine and Hidemitsu Kobayashi. 2018. Specific Antiproliferative Activity against Several Human Cancer Cells Possessed by *Cordyceps militaris* Grown in Viable Pupa of Silkworm Raised under Sterile Environment. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 988-997.
doi: https://doi.org/10.20546/ijcmas.2018.702.122