CHAPTER 32

ANTITUMOR COMPONENTS OF *COLLYBIA*

Byong Kak Kim, Sook Hee Kim and Eung Chil Choi

College of Pharmacy, Seoul National University, Seoul 151-742, Korea.

1. INTRODUCTION

Although most of the mushrooms that belong to the genus *Collybia* are edible, they have never been extensively studied for their metabolites. Among the species that are indigenous to Korea, *Collybia confluens* (Fr.) Kummer is fairly common. As part of our research project, we selected to examine the components of this mushroom. When it was examined for pharmacological activity, it showed anititumor activity. In this paper we report the antitumor components and their effects on the immunity of mice.

2. METHODS

2.1. Separation of Antitumor Components

After the mycelia of *Collybia confluens* were grown in a fungal medium of glucose, peptone, yeast extract and seven mineral salts on a rotary shaker, about 3.1 kg (wet weight) of the mycelium were harvested from 50 liters of the culture broth. The high molecular weight components of the mycelia were extracted according to the method of Kim *et al.* (1992). These components of Fraction A were further purified by DEAE cellulose column chromatography and were separated into Fractions B and C. Fraction C was named collyban and was separated by Sephadex G-200 gel filtration into Fractions C-I and C-II.

2.2. Antitumor Test

The tumor cells of S180 were inoculated into the left groin of ICR mice. Those mice in which the solid form of the tumor cell grew were used in the antitumor test. These mice were divided into two groups: control and treated. Two different doses of each fraction were injected daily into the treated group for 10 days, whereas the control group was injected only with saline. After 30 days, the tumors were excised and weighed. The inhibition ratio was calculated by comparing the tumor

weights of the control and treated groups.

After HeLa cells were inoculated into nude mice of BALB/C strain, respective doses of 50 mg and 100 mg/kg/day of collyban were injected into the mice for seven days. The tumor volumes were measured and compared to calculate the inhibition ratio.

2.3. Effects of Antitumor Components on Cancer Cells

To clarify the mechanisms underlying the antitumor activity of the components of the edible mushrooms, their direct cytotoxicity on cancer cells was examined. For this test, S180, P388, L1210 and HeLa cell lines were used (Carmichael *et al.*, 1987).

2.4. Effects of Antitumor Components on Immunity

When macrophages are activated, they release superoxide anion, which can be measured by using colorimetry. The coloration is achieved by glucose, ferricytochrome C and opsonized zymosan. Effects of collyban on macrophage activation were compared according to the method of Richard *et al.* (1975).

Since macrophages also release acid phosphatase when activated, the degree of the activation by collyban was measured according to the method of Suzuki (1989). The effects of collyban on the activity of B lymphocytes were examined by using sheep red blood cells (SRBC) and ICR mice according to the method of Kwag *et al.* (1992). Effects of collyban on delayed-type hypersensitivity (DTH) were evaluated by using SRBC and ICR mice according to the method of Kwag *et al.* (1992). The influence of collyban on natural killer (NK) cells was assessed by the method of Kiessling *et al.* (1976).

3. RESULTS

The tumor inhibition ratios of the five fractions ranged from 46% to 75% against the solid forms of sarcoma 180 in ICR mice at doses of 20 and 59 mg/kg/day when given intraperitoneally. Fraction C especially, which was named Collyban, exhibited a marked life-prolonging effect against ascitic forms of sarcoma 180 at a dose of 50 mg/kg via intraperitoneal administration. To extend the spectrum of the antitumor activities and eliminate the effects of allograft rejection, characterization of the antitumor effects of Collyban was performed in syngeneic host-tumor systems. Collyban did not show antitumor activity against L1210 murine leukemia in CD2F1 mice but prolonged their life span against ascitic forms of MM46 carcinoma in C3H/HE mice. Also, it exhibited antitumor activity against human cervical cancer HeLa cells that were xenografted into nude mice having BALB/c genetic backgrounds following intraperitoneal injection at a dose of 100 mg/kg/day.

When Collyban was examined for immunological activity to elucidate the mechanism of its antitumor activity, it did not show any direct cytotoxicity against S180, P388 and L1210 murine tumor cells except the direct action on HeLa cells. Collyban increased the number of peritoneal exudate cells of mice, especially the mature forms of polymorphonuclear leucocytes. From flow cytometric analysis, Collyban apparently induced the increase of Mac-1 positive murine spleen cells in the early stage of tumor growth. Moreover, at the late stage, it restored the decreased percentages of CD4 and CD8 positive spleen cells in the tumor-bearing mice to normal levels.

Collyban increased the production of acid phosphatase and the superoxide anion released from

activated macrophages in mice. It showed the adjuvant effect by increasing the number of the hemolytic plaque forming cells against sheep red blood cells, and also increased the production of alkaline phosphatase which was an index of the proliferation of B lymphocytes in the murine spleen cells. In cellular immunity, Collyban restored the depressed response of delayed type hypersensitivity in the tumor-bearing mice to the normal level. In nude mice, Collyban potentiated the cytotoxicity of natural killer cells which are one of the important nonspecific effector cells.

In order to characterize the antitumor components, Collyban was examined by chemical analysis. It comprised acidic protein-bound polysaccharides composed of 31% polysaccharide, 27% protein and 3% hexosamine. Collyban was fractionated into two fractions, Fr. C-I (M.W. : 500 KD) and Fr. C-II (M.W. : 30 and 80 KD) by Sephadex G-200 gel filtration chromatography.

Collyban showed a direct effect on HeLa cells, activated various immune effector cells and restored the decreased immune response of the tumor bearing host. Therefore, it was concluded that Collyban acts as an immunomodulator.

REFERENCES

CARMICHAEL, J., DEGRAFF, W.G., GAZDER, A.F., MINNA, J.D. & MITCHELL, J.B. (1987). Evaluation of a tetrazolium-based semiautomated colorimetric assay, assessment of chemosensitivity testing. *Cancer Research* **47**, 936-942.

KIESSLING, R., PERTANY, G & KLEIN, G. (1976). Non-T cell resistance against a mouse moloney lymphoma. *International Journal of Cancer* **17**, 275-284.

KIM, B.K., CHUNG, H.S., CHUNG, K.S. & YANG, M.S. (1980). Studies on antineoplastic components of Korean basidiomycetes. *Korean Journal of Mycology* **8**, 107-113.

KIM, B.K., ROBBERS, J.E., CHUNG, K.S., CHUNG, H.S. & CHOI, H.S. (1982). Antitumor component of *Cryptoporus volvatus*. *Korean Journal of Mycology* **10**, 111-117.

KIM, B.K., KWUN, J.Y., PARK, Y.I., BOK, J.W. & CHOI, E.C. (1992). Antitumor components of the cultured mycelia of *Calvatia craniformis*. *Journal of Korea Cancer Association* **24**, 1-18.

KWAG, S.D., BOK, J.W., HYUN, J.W., CHOI, E.C. & KIM, B.K. (1992). Studies on constituents of higher fungi of Korea (LXXIII). Antitumor components of the cultured mycelia of *Paxillus atromentosus*. *Korean Journal of Mycology* **20**, 240-251.

LEE, C.O., CHOI, E.C. & KIM, B.K. (1987). Immunological studies on the antitumor components of *Lyophyllum decastes*. *Journal of Korea Cancer Association* **19**, 57-62.

RICHARD, B.J., BERNARD, B.K. & HARD, P.M. (1975). The role of superoxide anion generation in phagocytic bacterial activity. *Journal of Clinical Investigation* **55**, 1357-1362.

SUZUKI, I., HARSHIMOTO, K., OIKAWA, S., SATO, K., OSAWA, M. & YADOMAE, T. (1989). Antitumor and immunomodulating activities of a β-glucan obtained from liquid-cultured *Grifola frondosa*. *Chemical and Pharmaceutical Bulletin (Tokyo)* **37**, 410-413.