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2011년 2월

석사학위논문

송이버섯(*Tricholoma matsutake*) 추출물이  
인체 구강암 KB Cell Lines에 미치는 영향

조선대학교 보건대학원

대체의학과

이 춘 식

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지도교수 문 경 래

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2010년 10월

조선대학교 보건대학원

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# ABSTRACT

## Effects of *Tricholoma matsutake* Extracts on the Human Oral Cancer KB Cell Lines

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The basic purpose of this study was to know whether two types of extracts from *Tricholoma matsutake* have or do not have the significant inhibitory effects on the cell viability of KB human oral cancer cell lines according to different molecular weights such chemoprevention as anticancer adjuvant and dietary factors.

This study was used two experimental methods of MTS assay and Western Blotting. MTS assay was performed to detect the inhibitory effects of *Tricholoma matsutake* extracts on the cell viability of the KB human oral cancer cell lines. Western Blotting was performed to observe the suppressive effects of *Tricholoma matsutake* extracts on inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression as cancer marker.

Methanol extracts from *Tricholoma matsutake* showed surprisingly the more than 80% suppression of iNOS expression and the 40% decrement of COX-2 expression with the progress of high consistency. Also they

showed about 65% inhibitive action on the cell viability rate with the progress of high consistency as a result of treatment for 48 hours.

Hot water extracts of *Tricholoma matsutake* showed the significant 60% suppression of iNOS expression at the low density of the log of 300  $\mu\text{g}/\text{ml}$ . This result means the potentiality of chemoprevention as anticancer adjuvant therapy because it occurred in the low density and the macromolecule above 40,000 KDa molecular weight.

As the conclusion of this study, the surprising suppression of iNOS expression and cell viability presented the high possibility of chemoprevention as anticancer adjuvant and dietary factors. but researches of activating substances such as the medicinal properties from *Tricholoma matsutake* as unsaturated fatty acid,  $\beta$ -glucans like Lentinan and Polysaccharide-K etc should continue for remedies.

Also the conclusion of this study can be stated the potentiality of chemoprevention on iNOS and COX-2 in inflammatory cells by dietary factors using such as food phytochemicals and polyunsaturated fatty acid.

Key words: *Tricholoma matsutake*, pine mushroom, Human Oral Cancer KB Cell Lines, MTS, Western Blotting, iNOS, COX-2

# 1. INTRODUCTION

Oral cavity cancer is a subtype of head and neck cancer. It is a cancerous tissue growth in the oral cavity. It may occur as a primary lesion originating in the oral tissues.<sup>1)</sup>

Oral cancers may start in the mouth tissues. There are many types of oral cancers. But squamous cell carcinomas is around 97%, adenocarcinoma and malignant melanoma is next.<sup>2)</sup> Most oral cancers are considered similar under the microscope. They are called squamous cell carcinoma. These tend to spread with the high rapidity.<sup>3)</sup>

All cancers are illness in the cells and are activated as a consequence of mutation of the DNA.<sup>4)</sup> The exact cancer cause is often undiscovered. The exact oral cancer cause is same in such meaning.<sup>5)</sup>

Smoking is associated with about 75% of oral cancer cases. Alcohol is another high-risk cause associated with oral cancer. Both a heavy smoker & drinker is considered to be a high synergistic effect on oral cavity cancer risk. Recent studies in Brazil and Germany indicate to alcohol-containing mouthwashes as being the cause of the oral cancer risk agents. <sup>6)</sup> Human papilloma virus (HPV) is a well-known risk cause and independent causative cause for oral cancer. There was a study in Sweden most recently that appeared at their current population of oral cancer patients and 60% in that group were HPV positive.

Korean National Cancer Center(KNCC) reported that approximately 160,000 Korean people are diagnosed with cancer in every year and 68,000 Korean people die of cancer. From 1983, cancer as

life-threatening disease became the first leading cause of death. 2,312 people are diagnosed with oral cavity cancer in Korea in 2007. but oral cancer mortality is very high, oral cancer death is fast increasing in Korean men especially.

In many Asian cultures chewing Piper betel, (Asian pepper plant - betel nut and dried leaves are chewed by southeast Asians) and Areca is considered to be a high risk cause for developing oral cavity cancer. In India (such practices are common) oral cancer exemplifies up to 40% of all cancers, contrasted to just 4% in the UK.<sup>7)</sup>

In the US oral cancer rates for about 8% of all malignant growths. Men are originated twice as women, particularly men older than from 40 years to 60 years. Recent data suggest that survival rates of oral cancer depend on the stage of the cancer at diagnosis. Overall, survival rates in all stages of initial diagnosis are around 50% at five years. Survival rates of stage 1 are 90%. We can know the emphasis on early detection to increase survival rate for patients.<sup>8)</sup>

Considering rates of out·break in USA, About 40 thousands persons are diagnosed with cancer of the mouth per a year. It will cause over 8 thousands deaths, killing roughly 1 person per 1 hour. Of those 40 thousands newly diagnosed persons, only slightly above 20 thousands persons will be alive in 5 years.

This survival rate has not significantly improved in decades. The death rate for cancer of the mouth is higher than any other cancers such as Malignant Neoplasm Of Stomach, breast cancer, liver cancer, colon carcinomas etc.<sup>9)</sup>

If we expand oral cavity cancers to include larynx cancer which the risk causes are similar, 50 thousands persons are diagnosed with these cancers per a year in the US alone. Over 650 thousands new persons in worldwide were found each year.

The death rate of oral cavity cancer is particularly high not because it is hard to discover or diagnose, but due to the cancer being routinely discovered late in its development. The first encounter oral cancer patients have up to a 20 times higher risk of developing a second cancer. After the first out·break, this heightened risk cause can maintain during ten years.<sup>10)</sup>

Hence considering that around 90% oral cancers are squamous cell carcinomas, a new therapy for treatment is needed such as special biotherapies based on the molecules and genes involved in the process. Apoptosis was specific to eliminate specific cancer cells. Targeted therapies can be approved the treatment for eliminating oral cavity cancers. Chemotherapy is useful in oral cancer cure with being used in combination with other treatments such as radiation therapy.<sup>11)</sup>

In the current research of biotherapies in the oral cavity cancer cure, many natural materials are adopted and investigated.<sup>12)</sup> But comparing to anticancer activity against Malignant Neoplasm Of Stomach, breast cancer,<sup>13)</sup> liver cancer, colon carcinomas<sup>14)</sup> etc have been conducted so many researches for their anticancer effects<sup>15)</sup> by using MTS assay,<sup>16)</sup> the study of the anticancer activities against oral cancer have been not enough in medicinal and food plants.<sup>17)</sup>

The current research reported that methanol extracts of some

medicinal plants<sup>18)</sup> revealed specially higher anticancer activity against oral cancer, they were such as; *Caesalpinia sappan*, *Anthriscus sylvestris*, *Rhus javanica*, *Curcuma aromatica*, *Inula helenium* etc.<sup>19)</sup>

But pine mushroom are not researched still now about anticancer effects against oral cancer.<sup>20)</sup> So this study is carried out with pine mushroom to verify whether it has chemoprevention properties or not as food and medicinal plants.

*Tricholoma matsutake* are hard to find and therefore the price is very expensive. The annual harvest of *Tricholoma matsutake* in Korea and Japan is very small, and the Korean and Japanese mushroom supply is largely imports from China, the North American and Northern Europe (Sweden and Finland).<sup>21)</sup>

So this study is carried out using China pine mushroom, they were easy to gain relatively and comparatively low price to buy for food plants.<sup>22)</sup> The most famous species in the East Asian<sup>23)</sup> are *Tricholoma matsutake*, also known as "song-i" in Korea and "matsutake" in Japan. *Tricholoma magnivelare* species that some are safe to eat in the North American known as "Pine mushroom", "ponderosa mushroom", "American matsutake".

In this study, KB (human oral epidermoid carcinoma cancer cell line) is compared after incubating in 2 types of extracts (hot-water extracts and methanol extracts). This experiment will present two consequences, the first is cell survival rate of oral cancer cell lines by MTS assay and the second is the suppression rate of Cox-2 and iNOS expression by western blotting.

## II. MATERIALS AND METHODS

### 1. Basic raw materials and extracting for experiments

The *Tricholoma matsutake* that was collected for this study was imported from Tibet in China by Geumgwang trading company in Beijing, China. *Tricholoma matsutake* was dried up in shade with cutting slices and was grinded into powder.

Two type extracts was extracted from *Tricholoma matsutake* powder for experiments. One is hot water extract, the other is methanol extract.

### 2. Reagents

The first degree of methanol reagent was used in this study. Reagents used in this study run as follows; LPS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2 H-tetrazolium inner salt (MTS), 1% penicillin and streptomycin were purchased from Sigma Chem. Co. in USA.

Dulbcco's Modified Eagle's Medium (DMEM) and 5% fetal bovine serum (FBS) were purchased from Gibco / Invitrogen (Grand Island, NY, USA)

ELISA Kit was purchased from Pierce endogen (Rockford, IL, USA) Microplate reader (MolecularDevices, Sunnyvale, CA, USA)

### 3. Cell culture & chemical treatment

Human Oral Cancer KB Cell Lines were purchased from American Tissue Culture Collection (Manassas, VA).

Human oral cancer KB Cell Lines were cultured in DMEM containing 5% fetal bovine serum (FBS) and 100 U/ml penicillin and 100 U/ml streptomycin in the atmosphere with 5% CO<sub>2</sub>.

### 4. Preparation of *Tricholoma matsutake*

Extracts of the powder of *Tricholoma matsutake* (100g) was extracted during 3 hours with distilled-deionized hot-water (1.3 liter) and lyophilized. Subsequently solvent was removed. The obtained powder was melted by concentration and then filtered with a twofold filter paper (Whatman NO.1) for use.

Another way was that the powder of *Tricholoma matsutake* (100g) was filtered during 48 hours after the methanol (1 liter) being poured. It was filtered out using a filter paper. Then it was extracted with evaporator which step was followed by cooling, lyophilization, and afterward got the reagent.

### 5. MTS assay for estimating cell viability

The effect of extracts of *Tricholoma matsutake* on cell viability were estimated according to the manufacturer's instructions of CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega, Madison, WI).



Human Oral Cancer KB Cell Lines were seeded in a 96-well plate and then incubated with different time (24H, 48H) dependent concentrations of extracts of *Tricholoma matsutake* and cell viability were observed closely.

MTS(3-(4,5-dimethylthiazol-20yl)-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium) solution quantified in an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 492 nm and 690 nm. The KB cell lines survival rate were expressed with percentages.

## 6. Western blot analysis (iNOS and COX-2)

Human Oral Cancer KB Cell Lines after processing with extracts of *Tricholoma matsutake* were harvested and disrupted. The protein supernatant fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to membranes and blocked with 5% skim milk followed by hybridization with the indicated antibodies.

Protein bands with horse radish peroxidase-conjugated secondary antibody were observed closely with Chemiluminescence Detection Kit.

## 7. Statistical analysis

Data are given expression as mean value  $\pm$  standard deviation of three independent experiments accomplished in triplicate at least. Data were computed for statistical significance using Student's t-test. The minimum level of statistical significance was set at  $p < 0.05$ .

### III. RESULTS

#### 1. Influence on the survival rate of cells

To identify whether the extracts from *Tricholoma matsutake* have or do not have cytotoxicity against oral squamous cell carcinoma KB cell lines, an MTS assay was conducted after treatment for 24 and 48 hours, according to different concentrations.

1) Effects of *Tricholoma matsutake* extracts on the KB human oral cancer cell lines viability using MTS assay (Methanol extracts)

To observe the cytotoxicity effects of the methanol extracts of *Tricholoma matsutake*, classified methanol extracts were used in treatments of Human Oral Cancer KB Cell Lines with the progress of the concentration (0, 300, 600, 900  $\mu\text{g}/\text{ml}$ ).

After being incubating for 24 and 48 hours with the methanol extracts of *Tricholoma matsutake*, the survival rate of the cells were measured.

In Figure 1, about 50% suppressive effect of the cell viability at the log of 600  $\mu\text{g}/\text{ml}$  was shown as a result of treatment for 48 hours. About more than 65% suppressive effect of the cell viability at the log of 900  $\mu\text{g}/\text{ml}$  was shown. It can be stated that the methanol extracts from *Tricholoma matsutake* are effective depending on concentration by MTS assay.

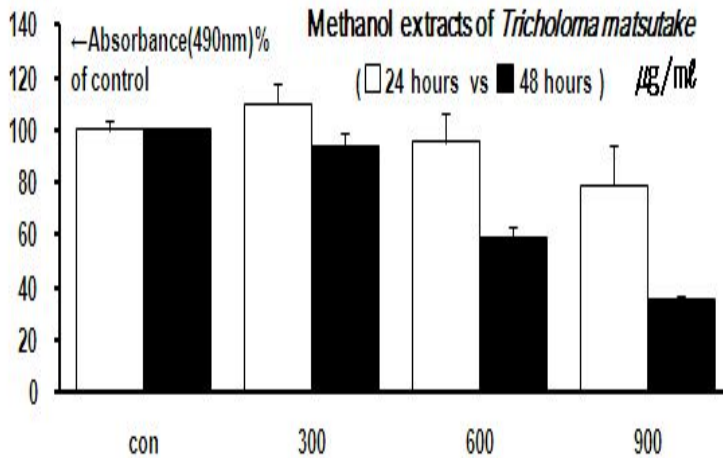


Figure 1. Effects of methanol extracts of *Tricholoma matsutake* on the inhibitory activity of the human oral cavity carcinoma KB cells of 24 and 48 hours after treatment with dependence on the concentration (0, 300, 600, 900 µg/ml).

## 2) Effects of *Tricholoma matsutake* extracts on the KB human oral cancer cell lines viability using MTS assay (Hot water extracts)

To observe the cytotoxicity effects of the hot water extracts of *Tricholoma matsutake*, classified hot water extracts were used in treatments of Human Oral Cancer KB Cell Lines with the progress of the concentration (0, 300, 600, 900 µg/ml).

After being incubating for 24 and 48 hours with the hot water extracts of *Tricholoma matsutake*, the survival rate of the cells were measured. However, there was non-significant effect as a result of the experiment in Figure 2.

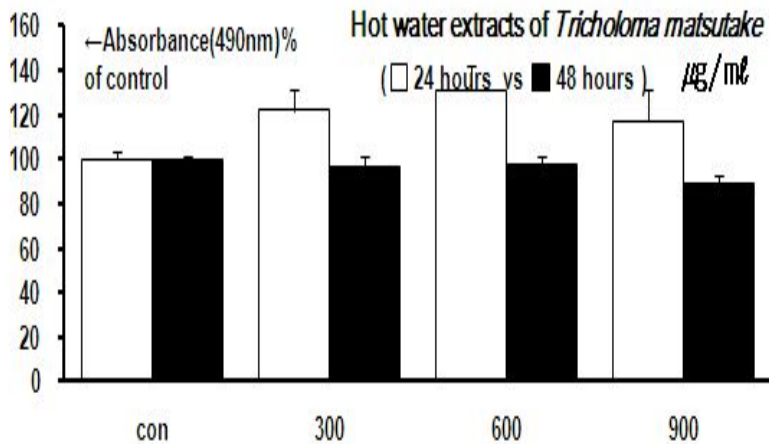


Figure 2. Effects of hot water extracts of *Tricholoma matsutake* on the inhibitory activity of the human oral cavity carcinoma KB cells of 24 and 48 hours after treatment with dependence on the concentration (0, 300, 600, 900  $\mu\text{g/ml}$ ).

## 2. Western blotting

1) Effects of methanol extracts of *Tricholoma matsutake* on cyclooxygenase-2 (COX-2) and Inducible Nitric oxide synthase (iNOS) expression in KB cells by Western Blotting.

To observe the inhibitory effects of classified methanol extracts of *Tricholoma matsutake*, after measuring house keeping gene  $\beta$ -actin as indicating non-interference in cellular mechanism, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions were compared with the control group and the test groups.

In the test groups, the expression of the iNOS was on the decrease

dramatically with the progress of the concentration (0, 300, 600, 900  $\mu\text{g}/\text{ml}$ ). In Figure 3, at the log of 600 and 900  $\mu\text{g}/\text{ml}$ , the expression of iNOS is observed surprisingly more than 80% suppression.

Also the expression of the COX-2 was shown the predisposition to dependence on the concentration. The expression of COX-2 is observed about 40% decrement with the progress of high consistency. (Figure 3)

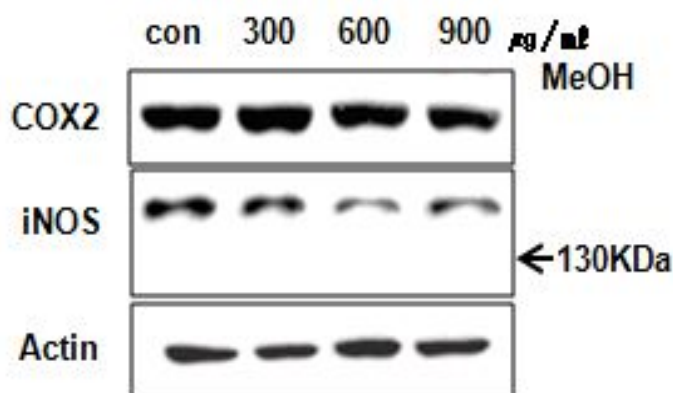


Figure 3. Inhibitory effects of classified methanol extracts of *Tricholoma matsutake* on the COX-2 and iNOS expression of the human oral cavity carcinoma KB cells.

2) Effects of hot water extracts of *Tricholoma matsutake* on cyclooxygenase-2 (COX-2) and Inducible Nitric oxide synthase (iNOS) expression in KB cells by Western Blotting.

Also according to the same procedures, to observe the inhibitory effects of classified hot water extracts of *Tricholoma matsutake*, after measuring house keeping gene  $\beta$ -actin as indicating non-interference in

cellular mechanism, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expressions were compared.

In the test groups, at the low density of the log of 300  $\mu\text{g}/\text{ml}$  in Figure 4, the expression of iNOS is observed about 60% suppression. This result was very significant because it occurred in the low density of 300  $\mu\text{g}/\text{ml}$  and the macromolecule above 40,000 molecular weight. This result means the potentiality of chemoprevention.

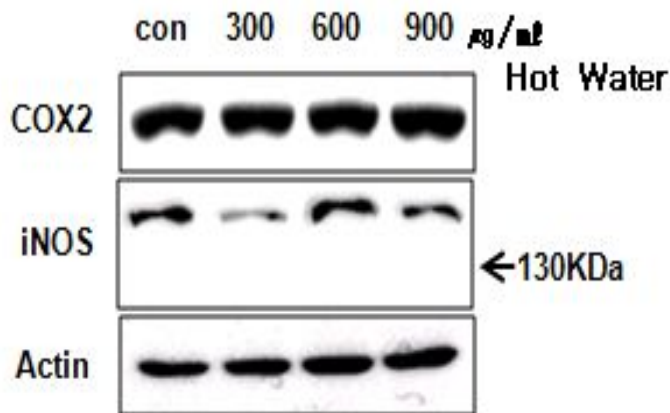


Figure 4. Inhibitory effects of classified hot water extracts of *Tricholoma matsutake* on the COX-2 and iNOS expression of the human oral cavity carcinoma KB cells.

## IV. DISCUSSION

*Tricholoma matsutake* (Korean: "Song-i", Japanese: Matsutake, Chinese: 松栉, English: Pine mushroom) is the mushroom that grows in Asia, North America and Europe. It is prized by Korean, Chinese and Japanese for its superb fragrance.<sup>24)</sup>

The habitate and distribution of *Tricholoma matsutake* is that it grow under trees and are commonly concealed under fallen leaves and it grow in duff on the forest floor. It forms commonly a symbiotic relationship with the roots of pine tree. *Tricholoma matsutake* are known to grow in Korea, China, Japan, Sweden, Finland, the North America.

Human uses of fungi for food or other purposes have a long history. The historical usage study and sociological impact of fungi is given a general description as the folk mycology. Many species are being developed for antibiotics, anti-cancer drugs and cholesterol-lowering drugs, vitamins. New methods have been developed recently for genetic engineering of fungi,<sup>25)</sup> being able to do metabolic engineering of many fungal species. For instance, genetic modification of yeast species.<sup>26)</sup>

This ways of pharmaceutical production are potentially more efficient than traditional production by the original source organisms.<sup>27)</sup> Many species produce metabolites that are major sources of pharmacologically active drugs. Particularly important are the antibiotics, including the penicillins, a structurally related group of  $\beta$ -lactam antibiotics that are synthesized from small peptides. Modern penicillins are semisynthetic

compounds, obtained initially from fermentation cultures, but then structurally altered for specific desirable properties.<sup>28)</sup>

Certain mushrooms are enjoyed with use as therapeutics in folk medicines, such as complementary alternative medicine and traditional chinese medicine. Notable medicinal mushrooms include such as following; *Agaricus blazei*,<sup>29)30)</sup> *Cordyceps sinensis*,<sup>31)</sup> *Ganoderma lucidum*.<sup>32)</sup> Research has identified compounds produced by many fungi that have inhibitory biological effects against viruses<sup>33)34)</sup> and cancer cells.<sup>35)</sup>

The shiitake mushroom is a material of lentinan, a clinical drug approved for usage in cancer treatments in many countries.<sup>36)37)</sup> In Europe and Japan, a chemical derived from *Trametes versicolor*, polysaccharide-K, is an recommended adjuvant for cancer therapy.<sup>38)</sup>

*Tricholoma matsutake* are enjoyed with use as food and therapeutics in folk medicines, but has not been researched yet as an adjuvant for oral cancer therapy such as both Complementary Alternative Medicine and Traditional Chinese medicine.

The essential part of the main properties of *Tricholoma matsutake* is unsaturated fatty acid (82.6~86.7% among fatty acid). Its potassium (K) is more 10 times than oyster mushroom, more 40 times than button mushroom, more 3 times than black mushroom. And it is rich in iron 10 times comparing other mushrooms.

*Tricholoma matsutake* in Korean market are graded 1, 2, 3 and 4 according to the scheme of their appearance presented by the National Forestry Cooperatives Federation.<sup>39)</sup> The major components for the



discrimination of raw matsutake was demonstrated such as like fumaric acid, succinic acid, leucine, alanine, threonine, the metabolites choline etc.

Considering bioactive compounds and medicinal properties, *T. matsutake*, (Ohnuma et al., 2000)<sup>40)</sup> have a number of sterols, antioxidant, free-radical scavenging activity and anti-inflammatory.

The research was performed about the inhibition of nitric oxide (NO) and the free radical-scavenging ability (FRSA) production by many extracts obtained from pine mushroom (Lim et al., 2007).<sup>41)</sup> the extracts of first-grade pine mushrooms contained more FRSA than the lower grades of pine mushroom. Pine mushroom was reported to be possessed of anti-tumor activity against the in vivo growth of mouse cord blood fibroblastic sarcoma (syngeneic fibrosarcoma). (Ebina et al., 2002)<sup>42)</sup>

The pine mushroom contains beta-glucans.  $\beta$ -Glucan is the dietary supplement for promoting weight loss.  $\beta$ -glucans can show some active function on insulin response.

$\beta$ -glucans like Lentinan isolated from mushrooms were recommended before 20 years in Japan for adoption as chemotherapy adjuncts. The recommendation of using  $\beta$ -glucans with cancer remedy was in the USA before acquirement with the FDA approval for cancer treatments. The polysaccharide of D-glucose monomers is the glucan molecule. Various beta-glucans are important for medicinal purpose.

$\beta$ -glucans' active ability to the immune system are called to "biological response modifiers".<sup>43)</sup>  $\beta$ -glucans is considered to improve

the immune system to activate without overacting.<sup>44)</sup>  $\beta$ -glucans also is considered to reduce LDL cholesterol and to go back to the former state on wound healing and prevent infections.

Polysaccharide-K is a protein-bound polysaccharide. It is the boosting agent of cancer immune system in some countries in Japan, China and Europe. It was under public agreement of an adjuvant for cancer treatment in Japan.

Polysaccharide-K from mushroom can suppress cancer development. It was proved anticancer activity in vitro,<sup>45)</sup> in vivo<sup>46)</sup> and in human treatments. Human treatments show fact that it can suppress cancer recurrence by using with an adjuvant<sup>47)48)</sup> and the mushroom can suppress various human cancer cells in vitro.<sup>49)50)51)</sup>

Considering the use of *Tricholoma matsutake* on clinical applications for cancer treatment,  $\beta$ -glucans and Polysaccharide-K have been used as an immunoadjuvant therapy for cancer since 1980, primarily in Japan. There is a large collection of research that demonstrates beta-glucans have anti-tumor and anti-cancer activity.<sup>52)53)</sup>

Chemoprevention is defined as the use of drugs, vitamins, or other agents. This use is to try to reduce the risk of cancer. Also this is to delay the development cancer or reduce recurrence of cancer.

Chemoprevention study in cancer prevention is a clinical trial. Chemoprevention researcher studies whether taking certain medicines can prevent cancer. and they researches to know which food supplements can prevent cancer and same as which vitamins and minerals can do. So chemoprevention study is called agent study.

In a mouse model study, tumors and liver metastasis<sup>54)</sup> were inhibited by beta 1,3 glucan in interferon gamma conjunction. In some studies, the effects of chemotherapy were enhanced by beta-1,3 glucans. In a cancer experiment, beta-1,3 glucans conjunction resulted in reduced cancer mortality in using a mouse model.<sup>55)</sup> In advanced gastric cancer patients, the conjunction with chemotherapy of the beta-1,3 glucans from shiitake mushrooms resulted in prolonged life survivals.<sup>56)</sup>

Typically, the destruction of cancerous tissue does not involve neutrophils, because these immune cells considers cancer cell as "self" rather than "non-self." Currently monoclonal antibodies and vaccines were involved in cancer immunotherapies. They stimulate the acquired immune response.<sup>57)</sup>

Chemoimmunotherapy is chemotherapy combined with immunotherapy. Chemotherapy makes the growth of cancer cells kill or slow by using different drugs and food supplements. Immunotherapy makes the anti-cancer ability of the immune system stimulate or restore by using treatments. Chemoimmunotherapy regimen can be commonly combined with the variety of food supplements.<sup>58)</sup> The technology contains wide applicability for cancer therapy. Each form of cancerous tumor cell is to match specific antigens on the cell surface, but some form of cancer cell are common to various types of cancer. Different antigens to tumor cells is targeted in different immunotherapies. For example, Mucin 1 is present on about 70% antigens of all cancer cell types.

According to the consequence of above consideration, *Tricholoma matsutake* is very superb food and highly useful medicinal plant being used for long times as medicines in many countries. Also it is known as

*Tricholoma matsutake* contains many useful active substances for a panacea to Pan-Asian people. But now in the cases of the clinical use of *Tricholoma matsutake*, there are not lots of proper investigation for verification.<sup>59)</sup>

So in this study, *Tricholoma matsutake* was carried out an experiment such chemoprevention as an adjuvant for oral cavity cancer chemoimmunotherapy using Human Oral Cancer KB Cell Lines.

The infiltration of oral cavity carcinoma is very powerful. Oral cavity carcinoma don't stimulate the particular sensation of symptoms except pain. So when it is found, it is not already the incipient stage of cancer in many cases.

Inducible nitric oxide synthases (iNOS) and Cyclooxygenase-2 (Cox-2) is researched to influence to activate carcinogens and proliferation of cancer cells. To achieve the effect in chemoimmunotherapy, iNOS and Cox-2 experiments are usually carried out essentially. Because the anti-inflammation is ahead usually of the anticancer. Therefore it should be observed whether the extracts of *Tricholoma matsutake* are effective in suppressing the multiplication of KB cells or not.

As the consequence of being measured by MTS assay and western blotting, inhibitory effects of classified methanol extracts of *Tricholoma matsutake* on the COX-2 and iNOS expression of the human oral cavity carcinoma KB cells was just splendid with the progress of the concentration (0, 300, 600, 900  $\mu\text{g}/\text{ml}$ ). At the log of 600 and 900  $\mu\text{g}/\text{ml}$ , the expression of iNOS is observed surprisingly more than 80% suppression. (Figure 3)

Inhibitory effects of classified hot water extracts of *Tricholoma matsutake*, at the low density of the log of 300  $\mu\text{g}/\text{ml}$ , was observed as the 60% suppression of the expression of iNOS. Because this result was very significant, it occurred in the low density of 300  $\mu\text{g}/\text{ml}$  and the macromolecule above 40,000 molecular weight. Therefore, these facts can be considered as *Tricholoma matsutake* has the high potentiality of chemoimmunotherapy food on oral cavity carcinoma.<sup>60)</sup>

## V. CONCLUSION

This study has the basic purpose to know whether extracts from *Tricholoma matsutake* have or do not have the significant inhibitory effects according to different molecular weights (especially a high molecular substance) of such chemoprevention as anticancer adjuvant and dietary factors.

So the cell viability rate in methanol and hot water extracts from *Tricholoma matsutake* is important and the suppression rate of iNOS and COX-2 expression is more important.

As results of this study, the surprising suppression of iNOS expression presented the high possibility of such chemoprevention as adjuvant and dietary factors. but researches of activating substances such as the medicinal properties from *Tricholoma matsutake* as unsaturated fatty acid,  $\beta$ -glucans like Lentinan and Polysaccharide-K etc should continue to use for remedies.

1. Methanol extracts from *Tricholoma matsutake* showed about 50% and 65% inhibitory effects of cell viability at the log of 600 and 900  $\mu\text{g/ml}$  with the progress of high consistency as a result of treatment for 48 hours. This result can be concluded that the methanol extract from *Tricholoma matsutake* are highly effective dependence on concentration and time.

2. Hot water extracts of *Tricholoma matsutake* showed non-significant inhibitory effects of cell viability as a result of the experiment. The survival rate of the cells were non-significantly reduced. This result can

be concluded that the hot water extracts from *Tricholoma matsutake* are not significantly dependence on concentration and time.

3. Methanol extracts from *Tricholoma matsutake* showed the more than 80% surprising suppression of iNOS expression at the log of 600 and 900  $\mu\text{g}/\text{ml}$  with the progress of high consistency as a result of treatment for 48 hours. This result can be concluded that the methanol extracts from *Tricholoma matsutake* are dramatically dependent on concentration. This result means the potentiality of such chemoprevention as adjuvant and dietary factors.

Also the 40% suppression of COX-2 expression is observed with the progress of high consistency. This result can be concluded that the methanol extracts from *Tricholoma matsutake* are dependent on concentration upon COX-2 expression.

4. Hot water extracts of *Tricholoma matsutake* were shown the 60% significant suppression of iNOS expression at the low density of the log of 300  $\mu\text{g}/\text{ml}$ . This result can be concluded that the hot water extracts from *Tricholoma matsutake* are significantly dependent on concentration.

This result means the potentiality of chemoimmunotherapy. Because the 60% significant suppression of iNOS expression occurred in the low density of 300  $\mu\text{g}/\text{ml}$  and the macromolecule above 40,000 Kda molecular weight.

Also this result means the potentiality of cancer chemoprevention<sup>61)</sup> on iNOS and COX-2 in inflammatory cells by dietary factors<sup>62)</sup> using such as food phytochemicals and polyunsaturated fatty acid.<sup>63)</sup>

## VI. KOREAN ABSTRACT

### 송이버섯(*Tricholoma matsutake*) 추출물이 인체 구강암 KB Cell Lines에 미치는 영향

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이 논문은 송이버섯을 분말로 갈아서 열수와 메탄올로 각기 다른 농도의 추출물을 만들고 시간을 달리하여 저분자량 상태와 고분자량 상태에서 인체구강암 KB 세포주의 세포 생존율과 암 표적인자의 발현량을 측정하여 암 예방을 위한 보조제 및 식이요소의 가능성을 알아보고자 했다.

이 연구에서는 MTS assay 와 Western Blotting의 두 가지 실험 방법이 사용되었다. 송이버섯의 열수 추출물과 메탄올 추출물이 각각 인체구강암 KB세포주의 세포생존율에 미치는 영향을 측정하기 위하여 MTS assay를 시행했으며, 암 표적인자 COX-2와 iNOS의 발현량 억제에 미치는 영향을 측정하기 위하여 Western Blot assay를 사용하였다.

송이버섯 메탄올 추출물은 농도가 높아짐에 따라 (900  $\mu\text{g}/\text{ml}$ ) 암 표적인자 iNOS 발현량을 놀랍게도 80%나 억제시켰고, COX-2 발현량도 40% 감소시켰다. 또한 시간이 경과함에 따라 48시간 만에 농도 900  $\mu\text{g}/\text{ml}$ 에서 암 세포 생존율을 65%나 억제시켰다.



송이버섯 열수 추출물은 300  $\mu\text{g}/\text{ml}$ 라는 저농도에서 암 표적인자 iNOS 발현량을 60% 억제 시켰다. 이 실험 결과는 40,000 KDa 이상의 분자량에서 300  $\mu\text{g}/\text{ml}$ 라는 저농도를 가지고 암 표적인자 억제라는 결과를 낸 것이므로 매우 유의적이며 더 적극적으로 추가적인 실험의 필요성을 보여주는 것이라고 사료된다.

이 연구의 결론으로서, 송이버섯 추출물은 암 표적인자 iNOS 발현량의 놀라운 억제능 및 시간과 농도에 따른 암세포 생존을 억제능을 보였다. 또한 열수 추출물의 유의적 억제 효과는 저농도에서 나타났으므로 식품과정을 통한 암 예방적 가능성을 보여 준 것이라고 해석됨으로 추가적인 IN VIVO 실험이 요구된다.

또한 송이버섯에서 추출될 수 있는 약용요소 - 즉 불포화지방산, 레티난과 같은 베타 글루칸, 폴리사카라이드 K와 같은 - 에 대한 추가적인 분리 정제 실험이 필요하다고 생각된다.

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## 저 작 물 이 용 허 락 서

본인이 저작한 학위논문에 대하여 다음과 같은 방법 및 조건하에 대학교에 저작권을 위임할 것을 서약합니다.

1. 인터넷 및 온라인 서비스와 아카이빙을 위하여 저작물의 내용을 변경하지 않는 편집상 혹은 포맷상의 변경을 통한 복제를 허락함
2. 저작물의 DB 구축과 인터넷을 포함한 정보통신망에 공개하여 논문 일부 또는 전부의 복제·배포 및 전송을 허락함
3. 저작물에 대한 이용 기간은 3년으로 하고 계약 종료 2개월 이내에 별도의 의사표시가 없는 경우 기간을 계속 연장함
4. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판 허락을 하였을 경우 1개월 이내에 소속 대학에 통보함
5. 배포, 전송된 학위논문은 이용자가 다시 복제 및 전송할 수 없으며 이용자가 연구 목적이 아닌 상업적 용도로 사용하는 것을 금함
6. 소속대학은 학위논문 위임 서약 이후 해당 저작물로 인한 타인의 권리 침해에 관하여 일체의 법적 책임을 지지 않을 것을 확인함
7. 소속대학의 협약기관 및 한국교육학술정보원에 논문 제공을 허락함

동의여부 : 동의(V) 조건부 동의( ) 반대( )

※ 조건부 동의 및 반대인 경우 사유 및 조건을 기재하여 주시기 바랍니다.

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논문명(국문)	송이버섯 추출물이 인체 구강암 KB 세포주에 미치는 영향		
논문주제분야	총류( ), 철학( ), 종교( ), 사회과학( ), 순수과학( ), 기술과학(V), 예술( ), 어학( ), 문학( ), 역사학( )		
학위구분	석사 (V) 박사 ( )		
초록기술언어	영어, 한글	논문 쪽수	41 쪽
한글초록	<p>송이버섯 메탄올 추출물은 농도가 높아짐에 따라 (900 <math>\mu\text{g/ml}</math>) 암 표적인자 iNOS 발현량을 놀랍게도 80%나 억제시켰고, COX-2 발현량도 40% 감소시켰다. 또한 시간이 경과함에 따라 48시간 만에 농도 900 <math>\mu\text{g/ml}</math>에서 암 세포 생존율을 65%나 억제시켰다.</p> <p>송이버섯 열수 추출물은 300 <math>\mu\text{g/ml}</math>라는 저농도에서 암 표적인자 iNOS 발현량을 60% 억제 시켰다. 이 실험 결과는 40,000 KDa 이상의 분자량에서 300 <math>\mu\text{g/ml}</math>라는 저농도를 가지고 암 표적인자 억제라는 결과를 낸 것이므로 매우 유의적이며 더 적극적으로 추가적인 실험의 필요성을 보여주는 것이라고 사료된다</p>		
주제어(국문)	송이버섯, 구강암, 세포생존율		
논문명(원문)	Effects of <i>Tricholoma matsutake</i> extracts on the Human Oral Cancer KB Cell Lines		
본문기술언어	영어		
초록(원문)	<p>Methanol extracts from <i>Tricholoma matsutake</i> showed surprisingly the more than 80% suppression of iNOS expression and the 40% decrement of COX-2 expression with the progress of high consistency. Also they showed about 65% inhibitive action on the cell viability rate with the progress of high consistency as a result of treatment for 48 hours.</p> <p>Hot water extracts of <i>Tricholoma matsutake</i> showed the significant 60% suppression of iNOS expression at the low density of the log of 300 <math>\mu\text{g/ml}</math>. This result means the potentiality of chemoprevention as anticancer adjuvant therapy because it occurred in the low density and the macromolecule above 40,000 KDa molecular weight.</p>		
주제어(원문)	<i>Tricholoma matsutake</i> , oral cancer, iNOS		