Nutrition Research and Practice (2007), 1(3), 180-183

Cytoprotective effect of polysaccharide isolated from different mushrooms against 7-ketocholesterol induced damage in mouse liver cell line (BNL CL. 2)

Joo-Shin Kim[1§], Hau Yin Chung[2] and Keun Na[3]

[1]*Kwangil Synthesis Plant Co. Ltd., Guro-Gu, Seoul 155-055, Korea*

[2]*Department of Biology, Food and Nutritional Sciences Programme, and Food Science Laboratory, The Chinese University of Hong Kong, Shatin, N. T., Hong Kong*

[3]*Pulmuone R & D Center, Seodaemun-Gu, Seoul 120-600, Korea*

Received July 10, 2007; Revised August 10, 2007; Accepted August 24, 2007

Abstract

Cytoprotective ability of polysaccharides isolated from different edible mushrooms was investigated on the 7-ketocholesterol-induced damaged cell line. Polysaccharide extracts from six different edible mushrooms-Flammulina velutipes, Peurotus ostreatus, Lentinus edodes, Agrocybe aegerita, Agaricus blazei, and Cordyceps militaris- were prepared by hot water extraction and alcohol precipitation. Cytoprotective ability was evaluated by measuring the viable cells of the normal embryonic liver cell line (BNL CL. 2) in the presence of 7-ketocholesterol. At 80 μg/mL of 7-ketocholesterol, cytotoxicity was very high with a loss of 98% of viable cells after 20 h of incubation. With the addition of 200 μg/mL of each polysaccharide isolate to the cell line containing 80 μg/mL of 7-ketocholesterol, polysaccharide isolates from both *Flammulina velutipes* and *Peurotus ostreatus* could significantly inhibit the 7-ketochoelsterol-induced cytotoxicity in the cells. But other polysaccharide isolates were not effective in inhibiting cell damage caused by the oxLDL-induced cytotoxicity.

Key Words: 7-ketocholesterol, cytotoxicity, mushroom, polysaccharide

Introduction

Cholesterol oxidation products (COPs) have received considerable attention due to the biological activities associated with human diseases. More than 60 products have been reported to be generated from cholesterol autooxidation (Smith, 1981). Among those COPs, 7-ketocholesterol oxidized at the C-7 position is a major oxidation product of cholesterol found in food and human. It is known to be more cytotoxic and atherogenic than cholesterol when ingested by laboratory animals and is the most potent inhibitor of cholesterol biosynthesis that is essential for cell function (Kim *et al.*, 2001; Lyons & Brown, 1999).

7-Ketocholesterol not only inhibits the cell growth but also significantly reduces the activity of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase, a regulatory enzyme involved in cholesterol biosynthesis (Kim *et al.*, 2001; Peng *et al.*, 1997). 7-Ketocholesterol has been identified as a main component of oxidized low-density lipoprotein (oxLDL) and is strongly cytotoxic to endothelial cells causing apoptosis which is a mode of cell death (Ghelli *et al.*, 2002). Gheilli *et al.* (2002) reported that the inclusion of cells with 7-ketochoelsterol caused aconcentration-dependent and time-dependent decreases in the number of viable cells.

Biologically active compounds found in mushroom have been known for their medicinal and nutritional benefits to human health. Many studies have reported that the various types of polysaccharide isolated from mushroom possess bioactive properties such as anti-tumor, immunological, anti-complementary, anti-inflammatory, anti-coagulant, and hypoglycemic activities (Kewon *et al.*, 1999; Woo, 1983). Mushroom polysaccharide extracts were also reported to have scavenging effects on superoxidesand hydroxyl radicals produced by reactive oxygen species (Lui *et al.*, 1995). Based on these, they could be useful candidates as effective, non-toxic substances with the free radical scavenging activities among other naturally occurring substances.

Even though several essential biological activities in the mushroom polysaccharide extracts have been known, studies on their cytoprotective activity on the oxysterol-induced damaged liver cells as a result of the cholesterol autooxidation have been limited. We hypothesized that polysaccharides isolated from mushrooms might inhibit ox-LDL-induced cytotoxicity in a normal liver cell line. Therefore, the purpose of this study was to evaluate the cytoprotective ability of polysaccharide extracts prepared from six different edible mushrooms on the cells which had 7-ketocholesterol-induced damages.

§ **Corresponding Author:** Joo-Shin Kim, Tel. 82-2-851-8383, Fax. 82-2-851-8384, Email. coffee670@naver.com

Materials and Methods

Materials

Six edible mushrooms (*Flammulina velutipes, Peurotus ostreatus, Lentinus edodes, Tricholoma matsutake, Agaricus blazei, and Cordyceps militaris*) were obtained from the local market (Kyungdong Market, Jegi-dong, Seoul). Normal embryonic liver cell line, BNL CL. 2, was obtained from the Korea Research Institute of Bioscience and Biotechnology. 7-Ketocholesterol was purchased from the Sigma Company (St. Louis, MO).

Extraction of mushroom-induced polysaccharides

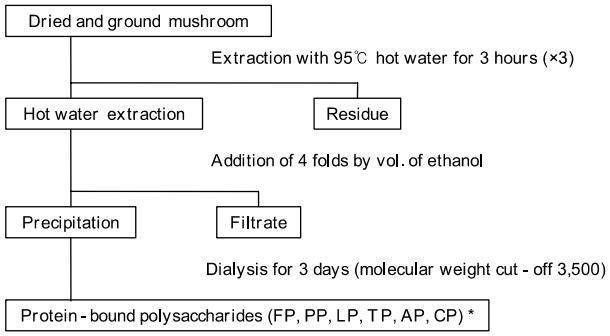

Fig. 1. Major steps to extract polysaccharides from mushroom. **Flammulina velutipes polysaccharides*: FP, *Peurotus ostreatus polysaccharides*: PP, *Lentinus edodes polysaccharides*: LP, *Tricholoma matsutake polysaccharides*: TP, *Agaricus blazei polysaccharides*: AP, *and Cordyceps militaris polysaccharides*: CP.

Polysaccharides from six edible mushrooms were extracted using the method based on Fig. 1 (Choi *et al.*, 1995).

Cell culture and cytotoxicity of 7-ketocholesterol

BNL CL. 2cells, normal mouse liver cell line, were used as a model system for testing the cytoprotective effect of the mushroom extracts. 7-Ketocholesterol (Sigma Co., St. Louis, MO) known to be strongly cytotoxic to various cell cultures was used as a cytotoxic substrate in a mouse liver cell line. To determine the optimal cell concentration and suitable cell density for plating, range of cells having seeding densities from 0 to 1×10^5 cells/well were screened at 540 nm for their absorbance. A seeding density of 5×10^4 cells/well was selected to determinate the cytoprotective effect of the mushroom extracts on the cell line. To investigate the cytotoxic effect of oxysterol on BNL CL. 2 cells, 7-ketocholesterol was added to the cells with concentrations ranging from 0 to 80 μg/ml and the cell density was determined after 20 h of incubation. Cytoprotective activity of the six different mushroom polysaccharide extracts was evaluated by measuring changes in the viable BNL CL. 2 cells in the presence of 80 ppm of 7-ketocholesterol at several specific periods (0, 8, 12, 14, 16 and 20 h).

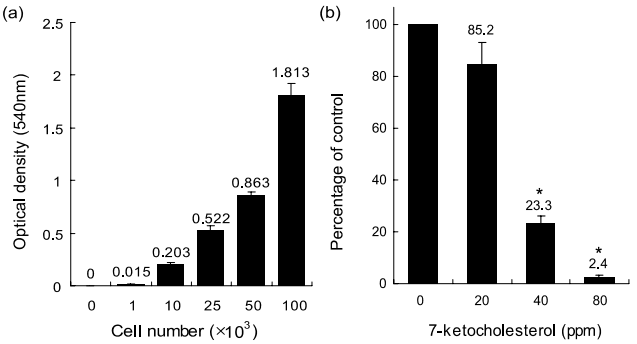

Fig. 2. (a) Absorbance for optimum cell number-response selection and (b) Viability of BNL CL. 2 cells after incubation for 20 h with 7-ketocholesterol at 20, 40 and 80 μg/ml. Results are expressed as mean ± SE (n=3) as cell survival measured at 540 nm, optical density of MTT. Statistical significant values between experiment and control groups were presented by an asterisk (* $p<0.05$).

Determination of cell viability

Cell density of the viable BNL CL. 2 cells was determined by the mitochondrial dehydrogenase activity using MTT 3- (4, 5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide reagent (Carmichael *et al.*, 1987) and the viable cell number was determined after 20 h of incubation by counting the nonstained cells using the trypan blue dye exclusion method (Sporri *et al.*, 2001). Cell viability was expressed as percentage of the control group which was not treated with polysaccharides.

Statistical analysis

All experimental and control groups were carried out in triplicates and all data were expressed as mean ± standard deviation (SE). To compare the difference between the experimental and the control groups, Student's t-test was performed and the statistical significance was indicated by an asterisk (* $p<0.05$).

Results and Discussion

The effect of 7-ketocholesterol on BNL CL. 2 cell proliferation

The optimal cell count was estimated to be 5×10^4 cells/well with cells evenly distributed, which corresponded to the absorbance of 0.863 at the wavelength of 540 nm (Fig. 2(a)). Addition of dimethylsulfoxide (1%), which was used to dissolve 7-ketocholesterol, did not significantly affect the growth of BNL CL. 2 cells (data not shown). Fig. 2(b) shows the growth pattern of BNL CL. 2 cells in the presence of 7-ketocholesterol. The proliferation of the cell line was decreased in a dose-dependent manner from high doses to low doses of 7-ketocholesterol. At the concentrations of 20, 40 and 80 μg/ml of 7-ketocholesterol, the viability of BNL CL. 2 cell was estimated to be 85.2 ± 8.1, 23.3 ± 2.7 and 2.4 ± 0.7% (n=3), respectively. With the addition of 7-ketocholesterol, the survival of the BNL CL. 2 cells was significantly inhibited as compared to the initial cell concen-

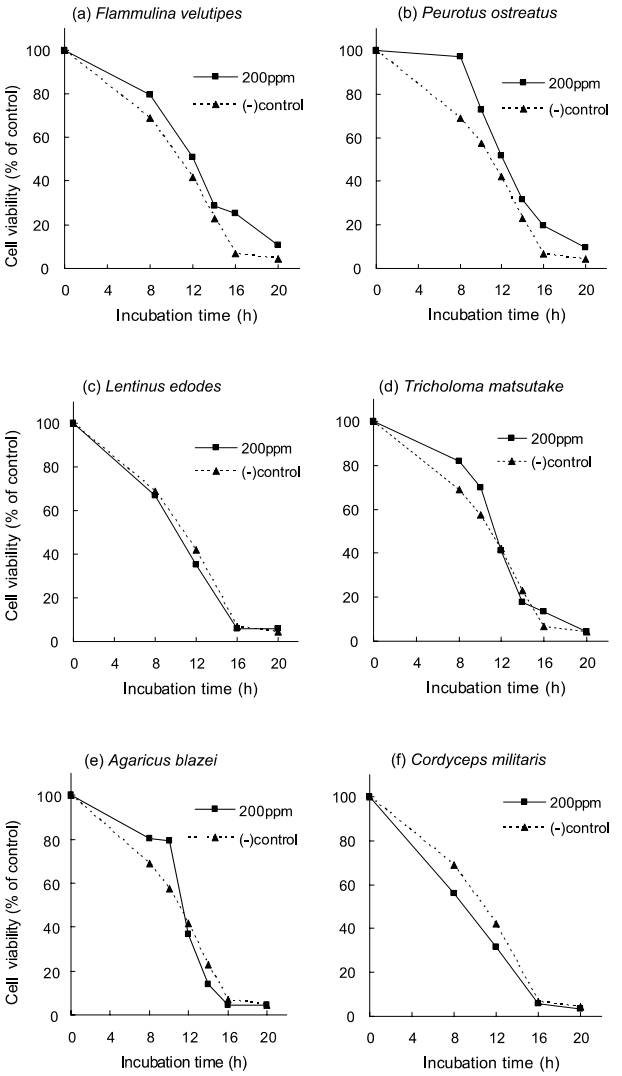

Fig. 3(a-f). Viability of the BNL CL. 2 cell culture treated with 80 μg/ml 7-ketocholesterol in the presence or absence of 200 μg/ml polysaccharide and incubated for 8, 12, 14, 16 and 20 h. Cell viability were expressed as % of cell survival relative to the control and based on the absorbance of the cells measured at 540 nm, optical density of MTT.

tration ($p<0.05$). Based on the results of the survival experiments, the cytoprotective effect of the different mushroom polysaccharides was determined using 80 μg/ml of 7-ketocholesterol on the BNL CL. 2 cells having a seeding density of 5×10^4 cells/well.

Cytoprotective effects of polysaccharide on 7-ketocholesterol induced cell death

Fig. 3(a~f) shows the cytoprotective activity of six mushroom polysaccharide extracts mixed with 80 μg/ml of 7-ketocholesterol. Concentration of less than 200 μg/ml extracts did not prevent cell death as a result of 7-ketocholesterol-induced cell damage (data not shown). At the extract concentration of 200

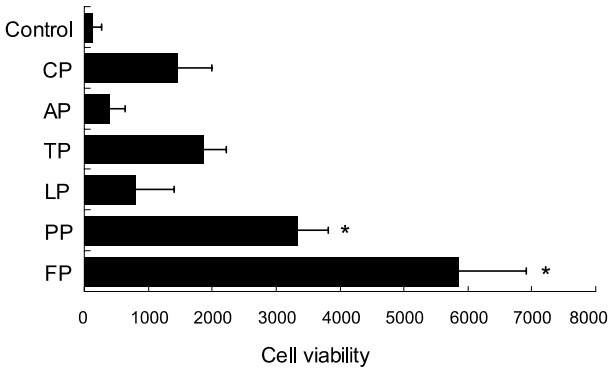

Fig. 4. Viable BNL CL. 2 cells in the presence of each 200 μg/ml polysaccharide from various sources. Cells were incubated for 20 h with 80 μg/ml 7-ketocholesterol added. Cell viability was measured by the trypan blue dye exclusion and expressed as mean ± SE (n=3). Statistical significance between experimental and control groups were denoted by an asterisk (* $p<0.05$). (CP: *Cordyceps militaris*, AP: *Agaricus blazei*, TP: *Tricholoma matsutake*, LP: *Lentinus edodes*, PP: *Peurotus ostreatus*, FP: *Flammulina velutipes*)

μg/ml, both samples of *Flammulina velutipes* and *Peurotus ostreatus* exhibited some cytoprotective effects. They significantly increased the number of viable cells compared to the control ($p<0.05$) when determined by the trypan blue exclusion method (Fig. 4). However, the rest of the mushroom extracts did not show any significant protective effect. Hence, the present study suggested that the polysaccharide extracts from both *Flammulina velutipes* and *Peurotus ostreatus* have significant cytoprotective effects to cells *in vitro* against damages caused by 7-ketocholesterol-induced cytotoxicity.

Literature Cited

Carmichael, J, DeGraff, WG, Gazdar AF, Minna JD & Mitchell JB (1987). Evaluation of a tetrazolium-based semiautomated colorimetric Assay: Assessment of chemosensitivity testing. *Cancer Res* 47:936-942.

Choi MY, Jung TY & Hahm KJ (1995). Cytotoxic effect of hot water soluble poly-saccharides from mushroom: Lentinus edodes and vitamin A & E supplementation against P388 cells. *Korean Journal of Nutrition* 28:1091-1099.

Ghelli A, Porcelli AM, Zanna C & Rugolo M (2002). 7-ketocholesterl and staurosporine induce opposite changes in intracellular pH, associated with distinct type of cell death in ECV304 cells. *Arch Biochem Biophys* 402:208-217.

Kewon MH, Jang H, Lim WJ, Chang H I, Kim CW, Yang HC, Hwang HJ & Sung HC (1999). Anti-complementary properties of polysaccharides isolated from fruit bodies of mushroom, *Pleurotus Ostreatus. J Microbiol Biotechnol* 9:450-456.

Kim J-S, Godber JS, King JM & Prinywiwatkul W (2001). Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran in aqueous model system. *J Am Oil Chem Soc* 78:685-689.

Liu F, Ooi VEC & Chang ST (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci* 60:763-771.

Lyons MA & Brown AJ (1999). Molecules in focus: 7-Ketocholesterol. *Int J Biochem Cell Biol* 31:369-375.

Peng SK, Tham P, Taylor CB & Mikkelson B (1997). Cytotoxicity

of oxidation derivatives of cholesterol on culturedarotic smooth muscle cells and their effect on cholesterol biosynthesis. *Am J Clin Nutr* 32:1033-1039.

Smith LL (1981). Distribution of autoxidaiton properties. In: *Cholesterol Autoxidation*, p.49-119. Plenum Press, New York and London. USA

Sporri S, Chopra V, Egger N, Hawkins HK, Motamedi M, Dreher E & Schneider H (2001). Effect of 5-aminolaevulnic acid on human ovarian cancer cells and human vascular endothelial cells in vitro. *Photochem Photobiol* 64:8-20.

Woo MS (1983). Studied on antitumor components of *Flammulina velutipes* of Korea (II). *Korean Journal of Mycology* 11:147-150.