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

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Abstract
Cytoprotective ability of polysaccharides isolated from different edible mushrooms was investigated on the 7-ketocholesterol-induced damage in 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-ketocholesterol-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 (Gheilli et al., 2002). Gheilli et al. (2002) reported that the inclusion of cells with 7-ketocholesterol caused acencentration-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.
Materials and Methods

Materials

Six edible mushrooms (Flammulina velutipes, Peurotus ostr-eatus, 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

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. 2 cells, 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 determine 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).

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-
Cytoprotective effects of mushroom polysaccharides 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 on the BNL CL.2 cells having a seeding density of 5 × 10⁴ cells/well. 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 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.

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