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Screening and Optimal Extraction of a New Antidementia β-Secretase Inhibitor-Containing Mushroom

Dong-Soo Seo[1], Eun-Na Lee[1], Geon-Sik Seo[2] and Jong-Soo Lee[1]*

[1]*Department of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735, Korea*
[2]*Korea National Agricultural College, Hwasung, Kyonggi-eo 445-893, Korea*

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To produce a potent antidementia β-secretase inhibitor from a mushroom, the β-secretase inhibitory activities of various mushroom extracts were determined. Methanol extracts of *Lentinula edodes* exhibited the highest inhibitory activity (40.1%). The inhibitor was maximally extracted when a fruiting body of *L. edodes* was treated with 50% methanol at 40°C for 24 h.

KEYWORDS : Antidementia, BACE1, β-Secretase inhibitor, *Lentinula edodes*

Dementia is thought to result from β-secretase-mediated precipitation of β-amyloid in the brain. Senile plaque β-amyloid is termed β-amyloid precursor protein (βAPP) (Haass and Selkoe, 1993). The amyloid precursor protein (APP) is processed into many different forms through a combination of cellular protease activities (α-, β-, and γ-secretase). β-Secretase is an aspartic protease also known as BACE1 (the β-site APP-cleaving enzyme). Its activity is the rate-limiting step in Aβ peptide production in vivo (Vassar *et al*., 1999). β-Secretase has maximal activity at an acidic pH, as agents that disrupt intracellular pH also inhibit β-secretase activity (Knops *et al*., 1995). β-Secretase is insensitive to pepstatin, an inhibitor of many types of aspartic protease (Sinha *et al*., 1999).

Many groups have attempted to identify β-secretase inhibitors using high-throughput screening of natural product extracts. Peptidic β-secretase (aspartic protease memapsin-2) inhibitor, OM 99-1, and other aspartic protease inhibitors such as OM99-2 and OM99-3 were developed from these efforts (Dorrel, 2000). In addition, non-peptidomimetic derivatives have been synthesized to optimize BACE1 inhibition (Garino *et al*., 2006). Recently, small-sized synthetic inhibitors containing a tetrazole ring and acidic heterocycle bioisosteres such as KMI-570, KMI-684, KMI-420, and KMI-429 were synthesized (Kimura *et al*., 2006). In contrast, efforts to discover naturally occurring BACE1 inhibitors have been comparatively limited. Several hydroxyl-containing inhibitors have been reported (Stachel *et al*., 2004). Catechins from green tea (Jeon *et al*., 2003), ellagic acid and punicalagin from pomegranate (Kwak *et al*., 2005), hispidin from mycelial cultures of *Phellinus linteus* (Park *et al*., 2004), octapeptide from *Saccharomyces cerevisiae* (Lee *et al*., 2007), and several compounds isolated from *Sanguisorbae radix* (Lee *et al*., 2005) have all been studied as BACE1 inhibitors. Although the treatment of Alzheimer's disease has been intensively studied, efficacious antidementia drugs or nutraceuticals lacking side effects have yet to be developed.

Mushrooms are nutraceuticals with health-stimulating properties and medical effects (Jeong *et al*., 2004; Kweon *et al*., 2002; Lee *et al*., 2003; Park *et al*., 2003; Yang *et al*., 2004). *Lentinula edodes* can be found in Korea, Japan, and China. Few studies have been done regarding the pharmaceutical effects of *L. edodes*, except for those concerning the cholesterol-lowering effect of eritademine (Kweon *et al*., 2002), antitumor activity of lentinan (Chung, 1982), and fibrinolytic effect of lentinacin (Chibada *et al*., 1969). Some commercial blood circulation enhancers as well as a number of antibiotics and immune-stimulating compounds that contain *L. edodes* have also been developed. The present study was performed to screen varieties of mushroom for a potent antidementia β-secretase inhibitor and to optimize the extraction protocol of the compound, with the aim of developing a novel antidementia β-secretase inhibitor that can be utilized as a drug or in functional foods.

The mushrooms used in this study were obtained from the National Institutes of Agriculture Science and Technology (Suwon, Korea) and the Korea National Agricultural College (Hwasung, Korea). Unless otherwise specified, all chemicals and solvents were of analytical grade. The recombinant human BACE1 assay kit was purchased from PanVera (Madison, WI, USA). Dried fruiting bodies (5 g) of mushrooms were pulverized and extracted with 200 *ml* each of water and methanol at 30°C for 12 h. The extracts were centrifuged at 10,000 ×g for 20 min and filtered through Whatman No. 41 filter paper. Each supernatant was lyophilized for analysis.

The BACE1 inhibitory activity assay was carried out

*Corresponding author <E-mail : biotech8@pcu.ac.kr>

according to the manufacturers protocol with previously described modifications (Byun *et al.*, 2005; Kwak *et al.*, 2005; Vassar *et al.*, 1999). A mixture of 10 μl assay buffer (50 mM sodium acetate, pH 4.5), 10 μl BACE1 (1.0 U/*ml*), 10 μl substrate (750 nM Rh-EVNLDAEFK-Quencher in 50 mM ammonium bicarbonate), and 10 μl of sample dissolved in the assay buffer was incubated for 60 min at 25°C in darkness. The mixture was illuminated at an excitation wavelength of 530 nm and light emitted at 590 nm was collected. The percent inhibition was determined by the following equation:

$$[1 - \{(S - S0)/(C - C0)\}] \times 100$$

where C denotes the fluorescence of a control (enzyme, assay buffer, and substrate) after 60 min of incubation, C0 is the fluorescence of the control at zero time, S is the fluorescence of the tested samples (enzyme, sample solution, and substrate) after 60 min of incubation, and S0 is the fluorescence of the tested samples at zero time. To check the quenching effect of the samples, the sample solution was added to the reaction mixture C, and any reduction in fluorescence by the sample was determined. The IC_{50} value was defined as the concentration of the BACE1 inhibitor required to reduce 50% of BACE1 activity. All data represent mean values of triplicate experiments.

To select the most potent BACE1 inhibitor-containing mushroom, various extracts from 80 species of mushroom fruiting bodies were tested for their BACE1 inhibitory activities (Table 1). Generally, methanol extracts showed higher BACE1 inhibitory activities compared to water extracts. Among the mushrooms tested, methanol extracts of the *Lentinula edodes* fruiting body showed the highest BACE1 inhibitory activity of 40.1%, and so was selected for further study. *L. edodes* has long been consumed because of its good rheological properties and nutrition. It also produces fibrinolyltic agents (Chibada *et al.*, 1969) and anticancer compounds (Chung, 1982). The present paper is the first to demonstrate that *L. edodes* produces a potent antidementia BACE1 inhibitor that may be useful medicinally and as a food supplement.

The effects of temperature on the extraction of the BACE1 inhibitor was investigated. Maximal extraction of the inhibitor was obtained at 40°C, with a BACE1 inhibitory activity of 39.5% evident at this temperature. Additionally, the BACE1 inhibitor was also extracted amply at 50°C and 70°C, with β-secretase inhibitory activities of 29.0% and 23.0%, respectively, evident. However, at temperatures below 30°C extraction was below 20% (data not shown). The optimum extraction temperature in the methanol-based procedure is lower than that methanol-based extraction of a platelet aggregation inhibitor from *Inonotus obliquus* (80°C) (Hyun *et al.*, 2006) but higher than that of *Pholiota adiposa* HMG-CoA reductase inhibitor (30°C) (Yu *et al.*, 2007).

Table 1. β-Secretase inhibitory activities of various extracts from fruiting body of the second-selected mushrooms

(unit:%)

Strains	Water extract	MeOH extract
Auricularia auricula-judae	1.5	not detected
Pleurotus eryngii	13.3	33.7
Flammulina velutipes	13.0	22.3
Lentinula edodes	18.3	40.1
Pleurotus ostreatus	not detected	16.6
Agaricus bisporus	8.7	18.3
Umbilicaria esculenta	not detected	11.4

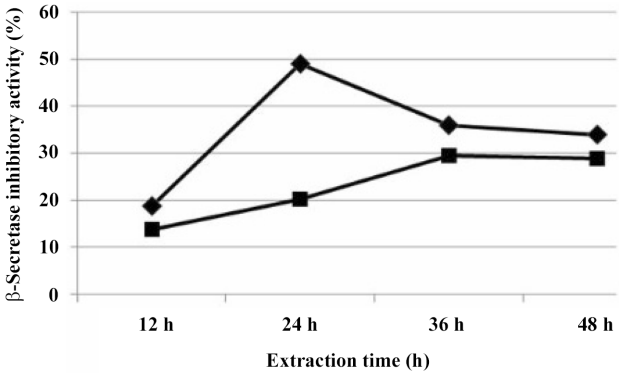

Fig. 1. Effect of extraction time on extraction of BACE1 inhibitor form *Lentinula edodes* by methanol at 40°C. ■: 80% methanol extraction, ◆ 50% methanol extraction.

The time course of the BACE1 inhibitor extraction was determined using 50% and 80% methanol. Inhibitor extraction increased as the extraction time increased. The maximal BACE1 inhibitor extraction levels was obtained by extraction after 24 h (Fig. 1).

The present results show that the optimal extraction condition for extraction of *L. edodes* BACE1 inhibitor is 50% methanol at 40°C with an extraction time of 24 h. Under these conditions BACE1 inhibitory activity is 48.5%.

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