Relation of Chitosan oligosaccharide-induced Melanin Production to The Activity and Expression of Tyrosinase in B16 Melanoma Cells

<u>Yoon Mi Yun</u>°, Cho Nam Young, Kim Kyung Won, Lee Ji Yun, Kim Chang Jong, Sim Sang Soo *College of Pharmacy, Chung-Ang University*

To investigate the effect of chitosi oligosaccharide on skin care, we measured tyrosinase activity and melanin production in B16 melanoma cells, and elastase and hyaluronidase activity. Chitosan oligosaccharide itself did not have any anti-oxidant activity in DPPH radical scavenging, and did not affect the proliferation of B16 melanoma cells. Chitosan oligosaccharide dose-depednetly increased mealnin production in the absence or presence of MSH. However, chitosan oligosaccharide did not have any influence on the tyrosinase activity and tyrosinase expression in B16 melanoma cells. These results suggest that chitosan oligosaccharide-induced melanin production may be independent on tyrosinase in B16 melanoma cells. On the other hand, chitosan oligosaccharide increased neutrophil elastase activity but decreased hyaluronidase activity in a dose-dependent manner. From the above results, chitosan oligosaccharide dose-dependently appears to increase melanin production in B16 melanoma cells and inhibit hyaruronidase activity, suggesting that chitosan oligosaccharide may be used as sun-tanning agent and water conservative agent.

[PB1-2] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Hypothetical Mechanisms of G protein-coupled neurodegeneration in glutamate excitotoxicity in human SH-SY5Y neuroblastoma cells

Nikolova Sevdalina°, Jin Da-Qing, Kim Jung-Ae

Department of Biotechnology Graduate School, Yeungnam University, Kyongsan 712-749, South Korea, College of Pharmacy, Yeungnam University, Kyongsan 712-749, South Korea

The cellular mechanisms by which excess exposure to the excitatory neurotransmitter glutamate can produce neuronal injury are unknown. In this study, we found that glutamate induced cell death at IC (50) of 100 microM on the cultured human SH-SY5Y neuroblastoma cells. It has been hypothesized that glutamate excitotoxicity is related with the elevation of calcium (Ca) levels. To determine the dependence of glutamate neurotoxicity on Ca environment, extracellular (EDTA) and intracellular (BAPTA/AM) chelator were used. Pretreatment with EDTA (1 mM) did not suppress the glutamate induced cell death. However, pretreatment with BAPTA/AM (2 μM) prevented glutamate-induced cell death. For further investigation the role of intracellular Ca homeostasis in mechanisms of neuronal cell death, SH-SY5Y human neuroblastoma cells were treated with A23187 calcium ionophore. Interestingly, we found that the combination of glutamate and A23187 (0.5 µM) considerably attenuates neuronal cell viability, compared to glutamate alone, indicating the pivotal role of Ca in the process of glutamate neurotoxicity. In the parallel experiment dantrolene (40 µm), a ryanodine receptor antagonist, was also found to prevent the glutamate-induced cell death. Results of other test suggest that N-methyl-D-aspartate (NMDA) receptors may play a role in mediating glutamate-induced lethal Ca influx. The lethal effect of glutamate was abolished by the selective NMDA-receptor antagonist (+)-MK 801 (10 µM). On the other hand, Pertussis toxin (PTX) blocked glutamate-induced cell death, indicating possible involvement of G proteins in the process. Moreover it has been reported that glutamate exerts its effect by both activation of ionotropic and metabotropic receptors (mGluR). In future we hope to establish whether activation of mGluR is also involved in glutamateinduced cell death. The effect of mGluR agonist trans-ACPD and antagonist (+)-MCPG will be examined.

Whitening Activity of Phenylpropanoid Compounds

Yoon Mi Yun^o, Park Young Mi, Lee Jin Hee, Kim Youn Joung, Kim Chang Jong, Sim Sang Soo College of Pharmacy, Chung-Ang University

To investigate the relationship between structure and biological activity of phenylpropanoids, we measured effects of phenylpropanoids on anti-oxidant and whitening activity. In DPPH radical scavenging activity, caffeic

acid analogues had anti-oxidant activity in a dose-dependent manner. Although phenylpropanoids did not inhibit purified tyrosinase activity, they significantly inhibited tyrosinase activity and melanin production in MSH-stimulated B16 melanoma cells. However, phenylpropanoids did not affect tyrosinase expression in MSH-stimulated B16 melanoma cells, which suggest that inhibition of MSH-induced melanin production was due to tyrosinase inhibition mediated via other signal pathways but not expression of tyrosinase. Phenylpropanoids also significantly inhibited both hyaluronidase and elastase activity, which suggests that phenylpropanoids may be used as whitening, water-conservative and anti-wrinkling agents. From the above results, phenylpropanoids appear to have anti-oxidant and whitening activity, particularly hydroxyl residue of aromatic ring plays an important role in antioxidant, whitening and water-conservative activity.

Effects of Cordyceps ophioglossoides extracts on the neuronal death and memory difficits

Park Byung Chul^o, Jin Da-Qing, Beak Sung-Mok, Lee Jae-Sung, Choi Hee-Don, Kim Jung-Ae Department of Pharmacy, Yeungnam University, Kyongsan 712-749, South Korea, College of Natural Resources, Yeungnam University, Kyongsan 712-749, South Korea, Korea Food Research Institute, Seongnam-si, 463-746, South Korea

We investigated whether the mushroom extracts can protect neuronal death and ameliorate memory deficits in Alzheimer"s disease induced by β -amyloid peptide[A β (25-35)]. Cellular model of Alzheimer"s disease was produced by using SK-N-SH human neuronal cells treated with Aβ. Treatment with 40uM Aβ for 48hours caused a 46% loss of cell viability. First, we examined the effects of 22 mushroom extracts on neuronal death using MTT assay. We found that 3 mushroom extracts increased viability of the cells from 46% to 87%. Especially, Cordyceps ophinoglossoides, one of 3 mushroom extracts, suppressed the generation of reactive oxygen species(ROS). Results from the in vitro experiments suggested Cordyceps ophinoglossoides contains effective ingradients which protect from Aß induced neuronal death. So, we examined the effect of Cordyceps ophinoglossoides on memory deficit in rats induced by AB. Initially the rats were given Cordyceps ophinoglossoides extracts were introperitoneally administered once a day for 3 weeks before A\beta injection. The rats were infused A\beta into the nucleus basalis using stereotaxic frame with Kofe microinjecter, and then they were given extracts of Cordyceps ophinoglossoides for two weeks until the water maze testing. The latency of Aβ-infused group was significantly long campared to untreated control group in the watermaze test. Cordyceps ophinoglossoides only-treated group did not change the latency of untreated control group. However, Cordyceps ophinoglossoides treated group significantly shortened the latency shown in Aβ-treated rats which was comparable to untreated control group. These results suggest that may be a good for prevention and treatment of Alzheimer"s disease.

[PB1-5] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Expression of taurine transporter and taurine uptake in mouse osteoblast cell lines Naomi Ishido1,2°, Emi Nakashima2, Yonug-Sook Kang1

1College of Pharmacy, Sook, myung Women's University, Seoul, Korea, 2Department of Pharmaceutics, Kyoritsu College of Pharmacy, Tokyo, Japan, 1College of Pharmacy, Sookmyung Women's University, Seoul, Korea

Taurine is present in a variety of tissue and exhibits many important physiological functions in the cell. Although it is known that many tissues mediate taurine transport, its functions of taurine transport in bone have not been identified yet. In the present study, we investigated the expression of taurine transporter (TauT) and taurine uptake using mouse stromal ST2 cells and osteoblast-like MC3T3-E1 cells, which is bone related cells. Detection of TauT mRNA expression in these cells were performed by reverse transcription polymerase chain reaction (RT-PCR). The activity of TauT was assessed by measuring the uptake of [3 H]Taurine in the presence or absence of TauT inhibitors. TauT mRNA was detected in these cells. [3 H]Taurine uptake was exhibited in these cells, which was dependent on Na $^+$, Cl $^-$ and Ca $^{2+}$, and inhibited by β -alanine and γ -amino-n-butyric acid. These results suggest that taurine has biological functions in bone and some effect on the bone cells.