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RAW264.7 macrophages에서 *Phellinus gilvus*로부터 분리된 Protocatechualdehyde의 nitric oxide 생성 억제

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Protocatechualdehyde, an antioxidant isolated from *Phellinus gilvus*, inhibits nitric oxide production in RAW264.7 macrophages

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Objectives

Protocatechualdehyde (PCA) is a water soluble antioxidant compound previously described in roots of *Salvia miltiorrhiza*, a traditional Chinese herb used to treat cardiac and vascular disease. In this study, we investigated in RAW264.7 macrophage cells the antioxidant activity and impact on nitric oxide (NO) production of PCA extracted from *Phellinus gilvus* (PG), a type of edible mushroom.

Materials and Methods

○ Materials

The fruiting bodies of PG used in this study were experimentally cultivated at Gyeongbuk Agricultural Technology Administration (Daegu, Korea), and grown rapidly for 3 months in artificial cultures.

PG were extracted with water (1:25) at 100 ℃ for 10 h, then the aqueous phase (Fa) concentrated at 80 ℃ in a rotary evaporator, and was then mixed with 95% ethanol (1:3, v/v) and stored at 4 ℃ overnight. The solution was then centrifuged at 15,000 rpm for 30 min, the precipitate dialyzed (1:100,000) in water, filtered, centrifuged, re-dissolved and lyophilized to obtain the polysaccharides (Fb). The supernatant was evaporated to remove the ethanol and extracted with ethyl acetate (4 vol.). The upper ethyl acetate layer (Fd) and lower aqueous phases (Fc) were then evaporated and lyophilized.

○ Methods

Fd fraction was analyzed using GC-MS and HPLC. The scavenging activity on 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals and inhibitory activity on NO production of PCA and Fd fraction was investigated.

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Results

PCA was identified in Fd fraction using GC-MS and HPLC analysis, and was approximately 8.66 ± 0.54 % of the Fd based on dry material mass. Treatment of RAW264.7 cells with increasing concentrations of PCA inhibited lipopolysaccharides-induced NO production and i-NOS and COX-2 mRNA expression in a concentration-dependent manner. The results also demonstrated that PCA possesses a strong DPPH scavenging activity, even higher than that of ascorbic acid. Treatment of RAW264.7 cells with various PG fractions did not affect cell viability. Our results suggest that PCA extracted from mushroom could potentially be used as an anti-inflammatory compound for disease treatment.

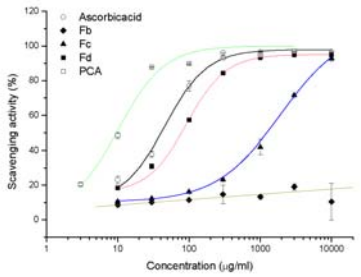

Figure 1. Comparison of DPPH free radicals scavenging activities among various fractions and PCA. Ascorbic acid was used as reference material.

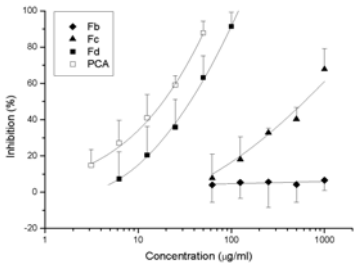

Figure 2. Inhibitory effects of various fractions and PCA on NO production. RAW264.7 cells were pretreated with different fractions or PCA for 30 min and then activated with LPS (0.5 μg/ml). After incubation for 24 h, nitrite amounts in medium were determined using Griess reaction. NO production of cells treated with LPS only was used as controls.

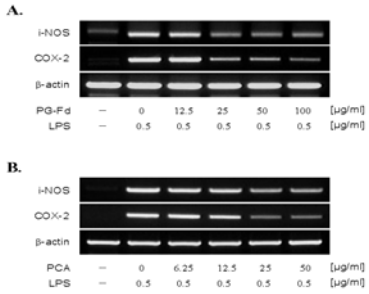

Figure 3. Effect of the Fd fraction and PCA on iNOS, COX-2 mRNA expression. RAW264.7 cells were pretreated with either Fd fraction (A) or PCA (B) at various concentrations for 30 min and then activated with LPS (0.5 μg/ml). After incubation for 8 h, iNOS and COX-2 mRNA were assessed by semi-quantitative RT-PCR.