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Protective effects of Kobophenol A from *Cargana sinica* on NO-induced-mitochondrial damage in human osteoblast-like MG-63 cells

Sungkyunkwan University: Jong-Hwan Kwak, Sung-Ryul Lee, Suhk neung Pyo;
Department of Herbal Medicine Resource, Kangwon National University: Sul-kyoung Park, Seon-A Jang, Eun-Hwa Sohn, Jung-Dae Lim*
Korea Institute of Science and Technology Information: Eun-Soo Sohn

Human osteoblast-like MG-63 cells에서 NO에 의해 유도되는 mitochondrial damage에 대한 골담초 (*Cargana sinica*) Kobophenol A의 보호효과

성균관대학교 : 곽종환, 이성열, 표석능; 강원대학교 : 박설경, 장선아, 손은화, 임정대*;
한국과학기술정보연구원 : 손은수

Objectives

Osteoblast death resulting from estrogen depletion is a problem occurred in many post menopausal women. The kobophenol A (Kob A) isolated from *Caragana sinica*, and we investigated the protective effects of Kob A MG-63 cells using SNP as a nitrosative stress model. We evaluated the protective effect of Kob A against NO-induced osteoblast apoptosis through mitochondrial damages and Kob A could be a promising treatment for diseases involved in osteoblast death.

Materials and Methods

○ Materials

Kob A [3,4'-Bibenzofuran]-6,6'-diol, 3'-(3,5-dihydroxyphenyl)-4-[(2S,3S,4R,5S)-4-(3,5-dihdroxyphenyl) tetrahydro-2,5-bis (4-hydroxy phenyl)-3-furanyl]-2,2',3,3'-terahydro-2,2'-bis(4-hydroxyphenyl)-,(2S,2'R,3S,3'R) extracted and purified from the dried roots of *Caragana sinica* <Lee et al., 2007>.

Kob A was identified through a comparison of its spectral data (NMR, IR, and UV)

○ Methods

1. **Cell culture :** Human osteoblast-like MG-63 cells were maintained in Dulbecco's modified Eagles medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (50 IU/ml), and streptomycin (50 mg/㎖)., 5% CO_2 at 37°C.
2. **Morphological observation of the cells**

- MG-63 cells culture (1 mM SNP, with or without Kob A, 0.4% Tryphan blueon), (24 h, 37°C)
- Hoechst 33342 (10 mg/㎖) staining and fluorescence microscopy
- Cytoplasmic and nuclear changes (PI staining)
- Cell fixing (3.7% formaldehyde<30 min>, methanol <10 min>)

† Corresponding author) : Jung Dae Lim, e-mail : ijdae@kangwon.ac.kr, Tel. : 82-33-570-6493

3. Mitochondrial membrane potential (MMP) was determined using Mitotracker red

- Changes in MMP(intracellular red fluorescent intensity) were analyzed by flow cytometry.
- Staining with 200 nM MTR for 30 min, the changes in the MMP level was observed by inverted fluorescence microscopy.

4. **Semi quantitative RT-PCR**

- Total RNA extraction : TRIZOL reagent (Invitrogen, Carlsbad, CA)
- PCR amplification : PCR Master (Promega, Madison, MA).
- GAPDH (Gene Bank No. NM_002046), Bcl-2 (Gene Bank No. NM_000633)

5. Western blottinvg analysis

- 10% SDS-polyacrylamide gel, transferred to an ECL membrane (Amersham, Arlington heights, IL), and probed with the appropriate antibodies.
- The blots were then developed using an ECL kit (Amersham, Arlington heights, IL)
- The intensities of the bands were quantified using densitometric software (IMageQuant, Amersham, Arlington heights, IL).

Results

1. Kob A alone had no effect on the cell morphology at any given concentration. In MG-63 cells exposed to SNP, there were significant morphological changes in the cytoplasmic and nuclear regions, whereas the co-treatment of SNP with Kob A had lesser morphological changes and cell death.
2. Kob A treatment also inhibited the SNP-incuced-lysosomal and mitochondrial membrane rupture.
3. Kob A recovered the expressionsof Bcl-2 and Bcl-XL proteins decreased by SNP. This suggests that Kob A exerts the protective effective against osteoblast apoptosis byincreasing the production of the anti-apoptotic proteins

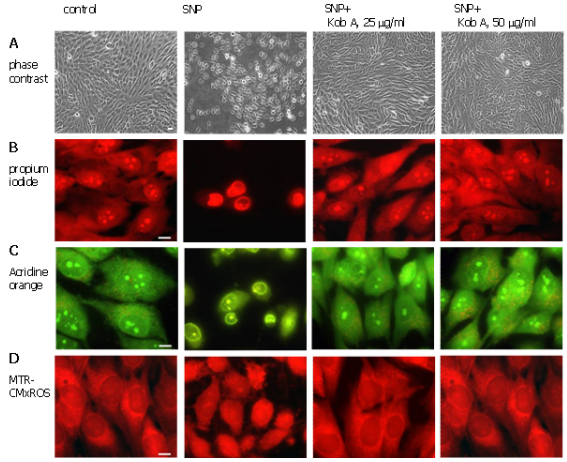

Fig. 1. Effects of Kob A on the SNP-induced nuclear and cytoplasmic changes. (A) cell shape (B) nuclear (C) lysosomal (D) mitochondria

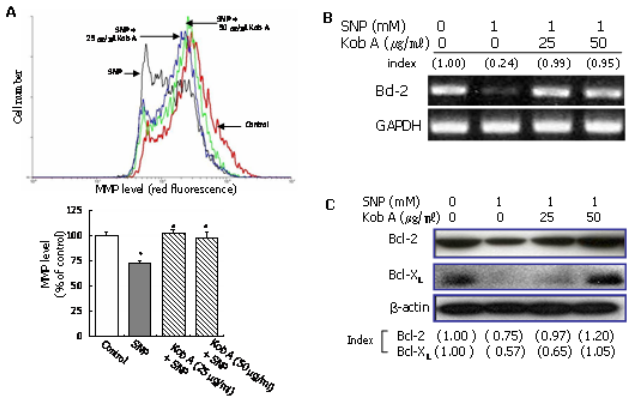

Figure 2. Effect of Kob A on mitochondrial damage induced by SNP (A) MMP, Anti-apoptotic mitochondrial protein level was measured by RT-PCR (B) and immnunoblotting (C).