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Kalopanaxsaponin A에의한 백혈병 세포주 U937에서의 Apoptosis 유도 및 작용기전에 관한 연구

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Kalopanaxsaponin A induces apoptosis in human leukemia U937 cells through extracellular Ca^{2+}influx and caspase-8 dependentpathways

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Objectives

Plants have been found to be a rich source of unique compounds that can induce apoptosis in premalignant or malignant human cells. As a part of our screening program to identify a natural compound with potential chemopreventive/ chemotherapeutic effect, we investigated the effect of kalopanaxsaponin A (KPS-A) , which was isolated from the stem bark of *Kalopanax pictus* Nakai (Araliaceae). It is of interest that the stem bark of this plant has been traditionally used for the treatment of rheumatoid arthritis, neurotic pain, and diabetes mellitus. However, active compounds for the therapeutical function are poorly studied. Thus, the present study was designed to evaluate 1) the effect of KPS-A on apoptosis of U937 human leukemia cells, 2) the mechanisms of KPS-A-induced actions in the cell growth.

Materials and Methods

○ Materials

The KPS-A used for this study was isolated from the stem bark of *Kalopanax pictus* Nakai (Araliaceae). Human promonocytic leukemia U937 cells were purchased from the Korean cell line bank and cultured in RPMI 1640.

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○ Methods

- Determination of DNA fragmentation and mitochondrial membrane potential
- Western blotting assay and measurement of $[Ca^{2+}]_i$

Results

Treatment of U937 human leukemia cells with 15 μM KPS-A for 1, 2, 4 and 8 h resulted in DNA fragmentation, DNA ladder formation, and an increase in the sub-G1 phase, suggesting the apoptotic activity. To further evaluate the induction of apoptosis, we investigated the involvement of caspases activation in KPS-A-induced apoptosis in U937 cells using Western blot analysis. Treatment with KPS-A stimulated a time-dependent cleavage of procaspase-3 and PARP, indicating the activation of caspase-3. Morever, the activations of both caspases-8 and -9 were evidenced by the degradation of their proenzymes. KPS-A stimulated Bid cleavage in a time-dependent manner, and that this coincided with changes in caspase-8 activation, suggesting that Bid is involved in cytochrome *c* release induced by KPS-A treatment. In addition, treatment with KPS-A dramatically decreased the cytosolic levels of Bax and increased its mitochondrial levels. Following treatment with 15 μM KPS-A for 10 min, $[Ca^{2+}]_i$ was found to be 2.3 times higher than that of the controls, and this elevated $[Ca^{2+}]_i$ was sustained for up to 30 min. Pretreatment with 5 μM EGTA(extracellular free Ca^{2+} chelator) for 5 min significantly suppressed the KPS-A-induced $[Ca^{2+}]_i$ levels, the sub-G1 phase, and the activation of caspases-3, -8 and -9. The anti-tumor or chemopreventive effects of kalopanaxsaponin A described in this work will contribute to potential therapeutical strategies for leukeminogenesis.

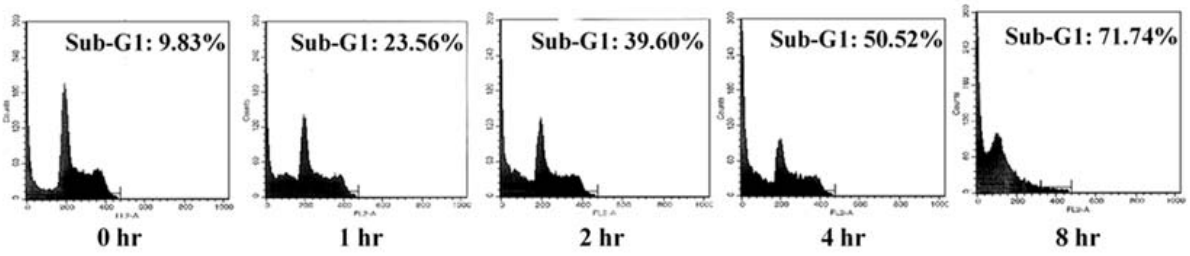

Fig. 1. Effects of KPS-A on apoptotic induction and DNA fragmentation in U937 cells

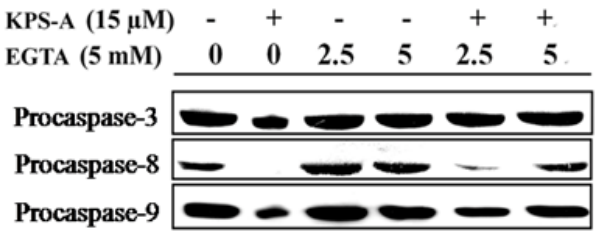

Fig. 2. EGTA attenuates KPS-A-induced Ca^{2+} influx, apoptosis, and caspase activation.