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ABSTRACT 218

Evaluation of the Effect of 6-shogaol on the Expression of *Insig1*, in Acute Lymphoblastic Leukemia cell line

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Our group has recently shown that ginger has strong anti-cancer activity against ALL cells. Moreover, it is reported that 6-shogaol (6Sh), a derivative of ginger, may bind to PPAR γ in cancerous cells and triggers its transcriptional activity. This project aims to evaluate the effect of 6-shogaol on the mRNA expression level of *Insig1*, the gene targeted by PPAR γ , in B-ALL cell line, Nalm-6, in order to investigate the mechanism by which it may inhibit leukemic cells.

Methods: 2×10^4 Nalm-6 cell line was treated with 6-shogaol (200 μ M) or solvent (0.4% DMSO) for 96h, and cell viability was determined using MTT assay. Furthermore, 12h after treatment with 200 μ M 6Sh or 0.4% DMSO, total RNA was extracted using TRIzol reagent and cDNA was synthesized. The effect of 6-shogaol on the expression of *Insig1* gene was assessed by the Real-time PCR technique. Briefly, 1 μ l (4ng/ μ l) of the synthesized cDNA was added to 9 μ l of the master mix solution (including 0.5 μ l of each primer, 4.7 μ l SYBR green Master mix, and 3.3 μ l Rnase free water). Each sample was analyzed and normalized to the level of the housekeeping gene (GAPDH). The threshold cycle values of the samples were calculated, and the gene transcription levels were analyzed using the $2^{-\Delta\Delta C_t}$ method.

Results: It was demonstrated, for the first time, that 6-shogaol had a significantly higher growth inhibitory effect on the 6Sh treated cells than their relative controls [$47.82 \pm 0.99\%$ vs 100%, respectively]. Moreover, the expression level of *Insig1* was increased in cells treated with 6-shogaol compared with the vehicle-treated cells by 1.55 ± 0.43 fold.

Conclusion: It is postulated that 6-shogaol may play its role against leukemic cells by increasing the expression levels of *Insig1* gene.

Keywords: Acute lymphoblastic leukemia, 6-Shogaol, PPAR γ , *Insig1*