

Genistein combined with L-asparaginase induced cell apoptosis in acute promyelocytic leukemia HL-60 Cells

Yin-Chen Hsiao, Jing-Gung Chung*

Department of Biological Science and Technology, China Medical University, Taichung 404, Taiwan

Abstract

Novel therapies such as the combination of two drugs acted more effectively than that of mono-therapy, however, genistein (Gen) and L-asparaginase (L-asp) underlying the synergistic cytotoxicity in HL-60 cells remains poorly understood. Gen, an isoflavone derivative, has been reported to inhibit DNA polymerases and topoisomerases II. More evidences have been confirmed that Gen changes various biological processes in estrogen-related malignancies such as breast and prostate cancers. L-asp is a chemotherapeutic agent used to remission acute lymphoblastic leukemia (ALL), almost 90% of children will survive the disease. The cure rates in adolescents, young adults, and adults have not kept pace with the improvements and associated with many adverse effects, such as coagulation abnormalities and cerebrovascular complications. So far the treatment of myeloid leukemia is not significantly effect and low sensitivity. Therefore, we focuses on combination of natural plant extracts Gen and leukemia clinical drug L-asp to investigate whether or not increase HL-60 cells. Because of the monotherapy Gen induced cell ROS release, ER stress occur and Ca^{2+} massive release in HL-60 cells. Western blotting used to examine the protein level which associated with apoptosis. Interestingly, after combination of the two drugs, the cell viability is synergic effect than that of monotherapy. Under treatment caused cell cycle arrest in G0/G1 phase and induced sub-G1 phase increase that was also confirmed by Annexin V assay. These result indicated that two drugs combination induced HL-60 cells apoptosis more sensitivity than L-asp only increased cell apoptosis.

Results

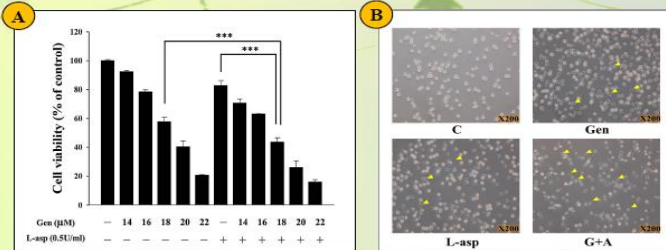


Figure 1. HL-60 cells after two drug combination (Gen and L-asp) treatment induced cell viability decrease than monotherapy. (A) Cells were treated with increasing of Gen or L-asp or in combination and the effects on cell viability were evaluated by flow cytometry. (B) After treatment the cell morphology changed and the arrows indicate cell death in each group. The values are the means \pm SD of three different experiments. *** p <0.001, compared to control.

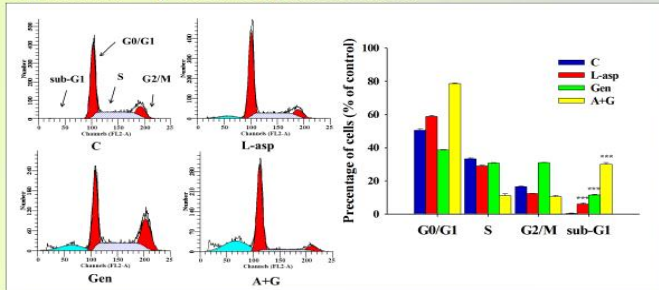


Figure 3. Combination treatment of gen and L-asp enhances cell apoptosis in HL-60 cells. HL-60 cells treated with gen or L-asp alone or in combination and cell cycle was measured. The values are the means \pm SD of three different experiments. *** p <0.001, compared to control.

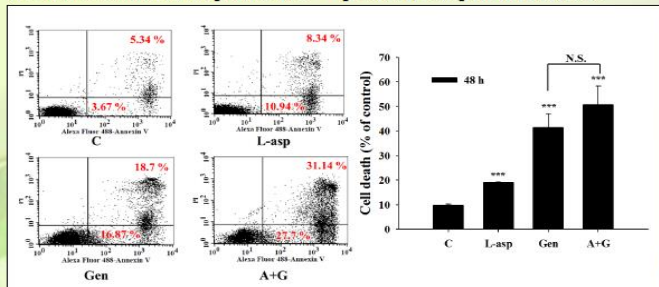


Figure 4. Combination treatment of gen and L-asp enhances cell apoptosis in HL-60 cells. HL-60 cells treated with gen or L-asp alone or in combination and apoptosis was measured. The values are the means \pm SD of three different experiments. *** p <0.001, compared to control.

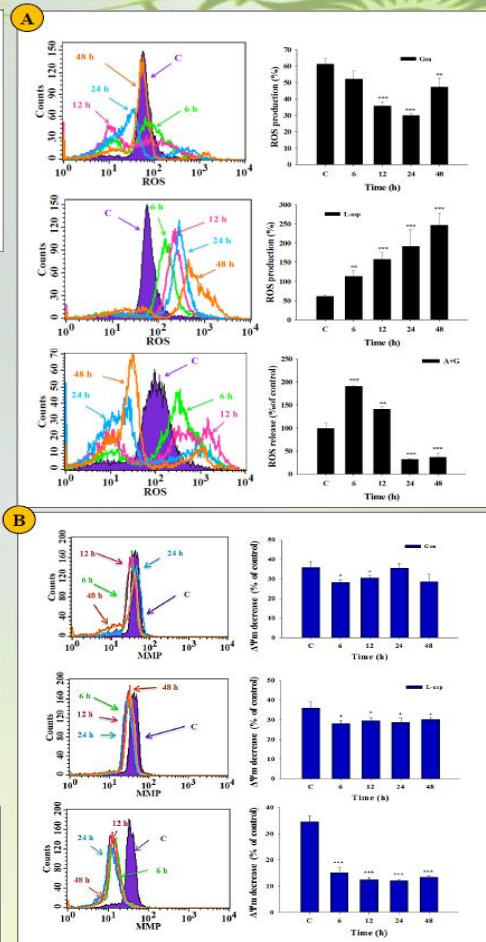


Figure 2. Effects of gen and L-asp alone or in combination of the ROS release decrease and MMP decrease after 48 hours. (A) Cells were treated with each group of gen, L-asp and gen plus L-asp, the ROS (reactive oxygen species) release were determined using flow cytometric analysis with time-dependent. (B) Cells were exposed with each groups of gen, L-asp and gen plus L-asp in time-dependent and the mitochondrial membrane potential (MMP) were determined. Both experiments were evaluated by flow cytometry assay. The values are the means \pm SD of three different experiments. * p <0.01, *** p <0.001, compared to control.

Conclusion

In conclusion, the results showed that after treatment of Gen and L-asp combination induced HL-60 cells morphology changed, cell apoptosis, MMP decrease, ROS release decrease, more effectively than mono-therapy.