

The effects of All- trans retinoic acid and DAPT (γ -secretase inhibitor) on gastric cancer cells.

Background: Gastric cancer is one of the most common and lethal malignancies with high mortality rate in the world. Aberrant activation of Notch signaling pathway may lead to malignancies including gastric cancer. Therefore, in recent investigations, Notch was proposed as a pharmacological target for the therapy of several cancers. Notch activation requires proteolytic cleavage by gamma-secretase. DAPT (N-[N-(3, 5 difluorophenyl -L-alanyl)] - Phenylglycine t-butyl ester) is a type of gamma-secretase inhibitor (GSI) that can efficiently block cleavage activity of γ -secretase. On the other hand, Retinoic acid and its derivatives (Retinoids) have been utilized as potential chemopreventive and chemotherapeutic agents due to their anti-proliferative, anti-oxidant, pro-apoptotic and differentiation effects. Currently, all-trans retinoic acid (ATRA) is an approved drug for the treatment of acute promyeloid leukemia (APL) patients.

Method and Materials: Human gastric carcinoma cells which are derived from (AGS) cell line were treated with different concentrations of DAPT (4, 5, 10, 15, and 20 μ M) and ATRA (2.5, 5, 10, 15, 20, and 25 μ M) and incubated for 72 hours. Cytotoxicity of these agents was examined through MTT assay. Ethidium bromide/acridine orange (EB/AO) staining was used for apoptotic cell detection. And finally, Caspase-3 and 7 activations were measured by luminescent kit and confirmed by RT-PCR technique.

Result: MTT assay results indicated that there was the highest mortality rate in 10 μ M DAPT & 25 μ M ATRA. Thus we continued our experiment with these two concentrations and combination of them. The EB/AO staining showed an increase in apoptotic cells after treatment with the combination of 10 μ M DAPT and 25 μ M ATRA. In addition, we indicated that the combination treatment of gastric cancer cells probably could induce apoptosis through Caspase3 and Caspase7 activation. Moreover, results of RT-PCR showed that in combination treated group, the expression of Caspase3 was increased in comparison with control group.

Conclusion: Combination of DAPT and ATRA had cytotoxic effects on AGS cells. This study provides the evidence that DAPT, ATRA and combination of them may induce cytotoxic effects on gastric cancer cell line by induction of apoptosis and it could be of value in realm of chemotherapy.

Key words: Gastric cancer, DAPT, ATRA, Apoptosis