The effects of All-trans retinoic acid on survival rate and expression profile of notch1 and hes1 genes in human gastric cancer (MKN-45 cell line)

Abstract

Background: Gastric cancer is one of the most common and lethal malignancies with high mortality rate in the world. 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 (APL) patients. Aberrant activation of Notch signaling pathway may lead to malignancies including gastric cancer. The aim of this project was to evaluate the possible effects of ATRA on viability, cell cycle alteration and induction of apoptosis of MKN-45 cell line. Some gene expression profiles of Notch signaling pathway were assessed as well.

Method and Materials: Human gastric carcinoma cells which are derived from (MKN-45) cell line were treated with increasing concentrations of ATRA (2.5, 5, 10, 15, 20 and 25 µM) and The viability of cells was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For apoptosis, caspase 3/7 activity was measured using Caspase-Glo 3/7 assay kit. Effect of ATRA on the distribution of cells in the different cell cycle phases was analyzed by Flowcytometry analyses after DAPI staining. Finally, hes1 and notch1 gene expression profiles were measured by RT-PCR technique.

Result: MTT assay results indicated that ATRA could reduces MKN-45 cell line viability, there was the highest mortality rate in 10 µM ATRA. ATRA exposure more than 10 µM had no significant effect on cancerous cells viabilities. Treatment of gastric cancer cells with ATRA could induce apoptosis through caspase3 and caspase7 activation. Flowcytometry findings showed the accumulation of MKN-45 cell line treated with ATRA in G1 phase of cell cycling. Moreover, results of RT-PCR showed that in ATRA treated group, the expression of notch1 was significantly reduced in comparison with control.

Conclusion: According to our findings, ATRA could exerts its cytotoxic effects on MKN-45 cell line through reducing cellular viability and inducing apoptosis. Cell cycle switch to G1 phase and reduction of notch1 gene expression provide the evidence of ATRA’s anti proliferative impact.
Key words: Gastric cancer, ATRA, Apoptosis, Notch signaling