The Effects of Cytotoxic Dose of Progesterone on Caspase 8 Activity Level in Colon Cancer (HT29) Cells

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***Abstract*—**It has been shown that progestins can induce apoptosis in cancer cells. We exerted this laboratory experimental research to assess the effects of cytotoxic dose of progesterone on caspase 8 activity in colon cancer (HT29 cells) in cell culture. Cytotoxic concentrations (0.01mg/ml) of progesterone was used in our study. HT29 cells were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm2 flasks, 96-well plates or 6-well plates. Activity level of caspase 8 was evaluated using calorimetric assay (405nm) through microplate reader. Analyses were conducted using the SPSS 20 and ANOVA. Our results indicated that exposure to 0.01mg/ml of progesterone led to significant increase in caspase 8 activity compared to control cells.

***Keyword*---** Progesterone, Caspase 8, HT29 Cells.

I. Introduction

The HT-29 cell line is the most widely used colorectal adenocarcinoma cell line with epithelial morphology, which is used in many cellular studies. These cells were first obtained in 1964 from 44-year-old Caucasian female tumor cells with colon adenocarcinoma. HT-29 cells under the standard conditions have a non-spontaneous growth, and they form undifferentiated cell lines that provide a very suitable model for investigating the effects of various factors on these cells. These cells are susceptible to 5-flaurouracil and oxaliplatin chemotherapy drugs, which are standard treatment options for colon cancer. [1-2]

Progesterone is essential for maintenance of pregnancy. It inhibits the contractions of smooth muscle myometritis, blocks the activity of uterine collagenase, and modifies the activity of the proteolytic enzyme. Progesterone is the most primitive hormone among all reproductive hormones. Observational studies and randomized trials have suggested that estrogens and/or progesterone may lower the risk for colorectal cancer. Inherited variation in the sex-hormone genes may be one mechanism by which sex hormones affect colorectal cancer, although data are limited. This is related to the presence of estrogen and progesterone receptors, with apparently higher concentrations in colon cancers than in adenomas. Epidemiological data and the finding of a significant reduction in colon cancer risk related to hormone replacement therapy (HRT), and in particular the length of HRT intake, indicate that progesterone/progestins have a preventive effect. [3.4]

Caspases are a family of endoproteases that provide critical links in cell regulatory networks controlling inflammation and cell death. The activation of these enzymes is tightly controlled by their production as inactive zymogens that gain catalytic activity following signaling events promoting their aggregation into dimers or macromolecular complexes. Activation of apoptotic caspases results in inactivation or activation of substrates, and the generation of a cascade of signaling events permitting the controlled demolition of cellular components. Activation of inflammatory caspases results in the production of active proinflammatory cytokines and the promotion of innate immune responses to various internal and external insults. Dysregulation of caspases underlies human diseases including cancer and inflammatory disorders, and major efforts to design better therapies for these diseases seek to understand how these enzymes work and how they can be controlled. [5,6]

Caspase-8 is a member of the cysteine proteases, which are implicated in apoptosis and cytokine processing. Like all caspases, caspase-8 is synthesized as an inactive single polypeptide chain zymogen procaspase and is activated by proteolytic cleavage, through either autoactivation after recruitment into a multimeric complex or trans-cleavage by other caspases. Thus, ligand binding-induced trimerization of death receptors results in recruitment of the receptor-specific adapter protein Fas-associated death domain (FADD), which then recruits caspase-8. Activated caspase-8 is known to propagate the apoptotic signal either by directly cleaving and activating downstream caspases or by cleaving the BH3 Bcl2-interacting protein, which leads to the release of cytochrome c from mitochondria, triggering activation of caspase-9 in a complex with dATP and Apaf-1. Activated caspase-9 then activates further "downstream caspases," including caspase-8. Knockout data indicate that caspase-8 is required for killing induced by the death receptors Fas, tumor necrosis factor receptor 1, and death receptor 3. Moreover, caspase-8-/- mice die in utero as a result of defective development of heart muscle and display fewer hematopoietic progenitor cells, suggesting that the FADD/caspase-8 pathway

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is absolutely required for growth and development of specific cell types. [5,7]

II. Material And Methods

Cytotoxic concentrations (0.01mg/ml) of progesterone was used in our study. HT29 cells were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Cells were grown and incubated in standard situation. Then, cells were sub-cultured into 75cm2 flasks, 96-well plates or 6-well plates. Activity level of caspase 8 was evaluated using calorimetric assay (405nm) through microplate reader. Analyses were conducted using the SPSS 20 and ANOVA.

III. Results

Our results indicated that exposure to 0.01mg/ml of progesterone led to significant increase in caspase 8 activity compared to control cells.

IV. Discussion

In our study, we found that exposure of colon cancer cells to cytotoxic dose of progesterone led to increased activity of caspase 8 enzyme. Caspase-8 is a cysteine protease that plays a pivotal role in the extrinsic apoptotic signaling pathway via death receptors. Therefore, progesterone induces apoptosis in colon cancer cells through extrinsic pathway.

In line with our findings, studies have shown that progestins have anticancer effects on various types of cancer cells, inducing apoptosis in cancer cell lines in vitro resulting in decreased proliferation rate in cells.

Research findings have shown that male and female sex hormones influence proliferation of cancer cells in different organs including prostate and other organs. Cellular and molecular studies have shown that sex steroid hormones act on genes expression by which can control apoptosis in target cells. [8] Human colon cancer cells are also affected by sex steroid hormones. In vitro and in vivo studies have revealed that sex steroid hormone including progestins have pivotal role in proliferation of colon cancer cells. [9] Antiproliferative effects of progesterone also has been demonstrated in a study in which colon cancer (HT29) cells proliferation is reduced when the cells are exposed to cytotoxic doses of progesterone. [10] The studies also have indicated that apoptosis in several types of cancer cells is associated with caspase 8 activity. [11] However, in contrast to our findings there are studies showing that progesterone has protective effect against apoptosis. [12]

V. Conclusion

In our study, we found that exposure of colon cancer cells to cytotoxic dose of progesterone led to increased activity of caspase 8 enzyme indicating that apoptosis occurred through extrinsic pathway in which caspase 8 is activated.

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