Journal of Pharmaceutical and Health Sciences 2016;4(3), 205-209.

Original Article

JPHS

The Exogenous Estrogen Antiproliferaive Effects On Cervical Cancer Cell Line: An In Vitro Study

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Abstract

Estradiol valerate (Exogenous estrogen) is a synthetic ester and is also a female estrogen hormone. There are studies showing that estradiol may have anticancer effects. This study was performed to reveal the effects of exogenous estrogens on the viability of cervical cancer cells in cell culture. We used Hela cells as our cell line in this study. Hela cells were exposed to 0.0001, 0.001, 0.01, 0.1, 1 and 10 mg/ml of estradiol. Control Hela cells were not exposed to estradiol. MTT assay was used to determine the viability of cervical cancer cells in cell culture. Results indicated that administration of 10 mg/ml Estradiol led to significant decrease in viability of Hela cells compared to control cells ($P<0.05$). Administration of 0.0001, 0.001, 0.01, 0.1 and 1 mg/ml of estradiol did not change the viability of Hela cells significantly compared to control group. The results are expressed as the mean ± SD of more than 3 independently performed experiments. Statistical significance was set at $p<0.05$. Motivated by the previous finding that consumption of steroid derivates correlated with cancer cell growth rate, According to the findings, high doses of estradiol have cytotoxic effects on cervical cancer cells. There are no studies revealing that estradiol may reduce the viability of cervical cancer cells.

Keywords: Estradiol, HeLa cell line, Viability, Antitumor

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Introduction

Estrogen hormone plays important role in sexual and reproductive development, mainly in women. They are also referred to as female sex hormones. The term "estrogen" refers to all of the chemically similar hormones in this group, which are estrone, estradiol, and estriol. Estrogens produced by the ovaries and, in lesser amounts, by the adrenal cortex, placenta, and male testes. Estrogen helps control and guide sexual development, including the physical changes associated with puberty. It also influences the course of ovulation in the monthly menstrual cycle, lactation after pregnancy, aspects of mood, and the aging process. Production of estrogen changes naturally over the female lifespan, reaching adult levels with the onset of puberty and decreasing in middle age until the onset of menopause. During the menstrual cycle, estrogen acts to produce an environment suitable for fertilization, implantation, and nutrition of the early embryo. Estrogens especially estradiol has an effect on target tissues by binding to fractions of cells called estrogen receptors. These receptors are protein molecules found inside those cells that are targets for estrogen action. Only estrogens (or closely related molecules) are able to bind to these receptors. Cervical cancer is cancer that starts in the cervix, the narrow opening into the uterus from the vagina. Human papillomavirus (HPV) is found in about 99% of cervical cancers. There are over 100 different types of HPV, most of which are considered low-risk and do not cause cervical cancer. High-risk HPV types may cause cervical cell abnormalities or cancer. More than 70 percent of cervical cancer cases can be attributed to 2 types of the virus, HPV-16, and HPV-18 often referred to as high-risk HPV types. In fact, by the age of 50, approximately 80% of women have been infected with some type of HPV. More than 12,000 women in the United States will be diagnosed with cervical cancer each year, and more than 4,000 of women will die. Possible symptoms of the more advanced disease may include abnormal or irregular vaginal bleeding, pain during sex, or vaginal discharge. Cervical cancer is the second most common type of cancer for women, but sinceit develops over time, it is also one of the most preventable types of cancer. There have been studies that local production of estrogen has also been detected in endometrial cancers and uterine leiomyomas. Studies have shown that estrogenic stimulation can influence cervical tumor genesis. It is known that aromatase is the key enzyme in the final step of estrogen biosynthesis and has a direct correlation with estrogen production, Hareesh B. Nair et al. Suggest that in situ aromatase expression may be a potential factor involved in the progression of cervical cancer. Findings by Rahim Ahmadi et al. showed that serum level of creatine kinase was significantly decreased in testosterone or estradiol receiving compared with control animals. Researchers found after 15 years of estrogen use increased the risk of breast cancer by 30%. Some of the researchers believed that estrogen promoted development of cervical cancer in cells infected with high-risk human papillomaviruses (HPVs). Researchers' results showed that chronic estrogen exposure was an essential cofactor required for the elaboration of squamous epithelial reproductive tract carcinogenesis by the HPV16 oncogenes. Aim of this study was to explore the relationship between estradiol and viability of cervical cancer cells in cell culture.

METHOD AND MATERIAL

A. Cell line

Hela cells, cervical cancer cell lines, were purchased from National Cell Bank of Iran (Pasteur Institute, Teheran, Iran) and were cultured in growth medium recommended

by the American Type Culture Collection. Reagents for cell culture were purchased from Cellgro (Herndon, VA). 4-Androstene-3,17-dione was purchased from Sigma-Aldrich (St. Louis, MO). The Cells were grown and incubated in a standard situation.

B. Protocol of Study

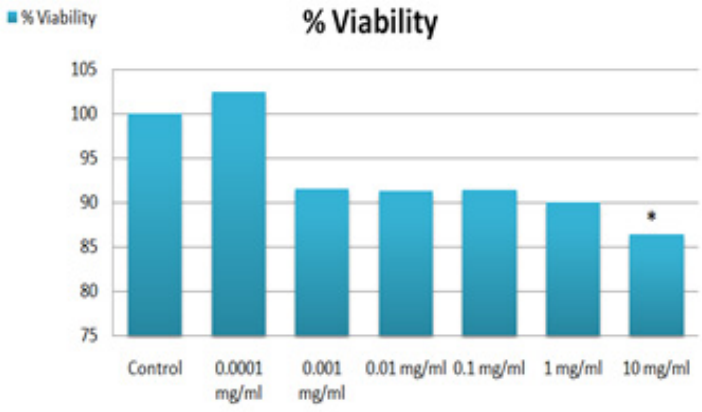
Different Concentrations (0.0001, 0.001, 0.01, 0.1, 1 and 10 mg/ml) of estradiol were prepared and used in our study. Cells were subcultured into 75 Cm2 flasks, 96-well plates or 6-well plates. The viability of different doses of the extract was assayed using MTT method. The MTT assay was a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may under defined conditions, reflect the number of viable cells present. Those enzymes were capable of reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which had a purple color. Other closely related tetrazolium dyes including XTT, MTS, and the WSTs, were used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With cell-impermeable WST-1, reduction occurs outside the cell via plasma membrane electron transport. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.

C. Statistical analysis

All values were presented as mean ±S.E.M. statistical significance was evaluated by One-Way analysis of Variance (ANOVA) using SPSS 20. Differences with $p<0.05$ were considered significant.

RESULT

Our results indicated that administration of 10 mg/ml of estradiol resulted in significant decrease in viability of Hela cells compared to control cells ($P<0.05$). Administration of 0.0001, 0.001, 0.01, 0.1 and 1 mg/ml of estrogen did not change the viability of Hela cells significantly compared to control group (Figure I).



** indicates significant difference compared to control group.*

Figure 1: Viability of Hela cells compared to control group.

DISCUSSION

Our finding shows that estradiol injection results in decreased viability of cervical cancer cells in highest dose (10mg/kg) in male and female rats compared to control animals. In contrast to this finding, there are studies showing that estrogenic stimulation has tumor genesis effect on cervical cancer. There are studies indicating that aromatase induction may lead to increased tumor growth and progression of cervical cancer. As we know, estrogen tends to induce aromatase expression in cells and stimulation of namely, cyclin D1, a cell cycle factor and proliferating cell nuclear antigen (PCNA), a marker of proliferation. It makes sense that aromatase subsequently cyclin D1 may lead to increase proliferation of cervical cancer cells in this study. It has also been shown that estrogen had promoted the development of cervical cancer in cells infected with high-risk human papillomaviruses (HPV). [27]

CONCLUSION

According to our finding, high doses of estradiol can reduce the viability of cervical cancer cells. It is proposed that the sexuality differences have no significant effect on the viability of cancer cells. Our finding showed that estradiol may result in decreased viability and proliferation of Hela cells. Since cancer cells are the major health problem, changes in its viability have serious effects on treatment and also creating a novel therapy for cancer patients. It also can be concluded that hormone replacement therapy imposes side effects on organs and tissues partly due to its effects on apoptosis and cell cycle and carcinogenic activities.

ACKNOWLEDGMENT

We appreciate all who helped us carry out this study.

Conflicts of Interest

None of the authors have any conflict of interest associated with this study.

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