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In Vitro Evaluation of the Effects of Zearalenone and α-Zearalenol on MCF-7 and MDA-MB-468 Cell Lines of Human Breast Cancer

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Abstract

Background: In the field of human breast cancer, the most recent researches referred to the influence of endogenous estrogen or exposure to environmental estrogen, as risk factors. Zearalenone (ZEN) as a mycotoxin and its derivative α- zearalenol (α-ZOL) are known nonsteroidal estrogenic compounds with potential endocrine disrupting properties.

Objectives: The present study was designed to investigate the effects of ZEN and α-ZOL on human breast cancer cell lines MCF-7 and MDA-MB-468.

Materials and Methods: Cell lines were treated by low and high doses of ZEN and α-ZOL (0, 1, 30, 62, 125, 250 and 500 ng/ml and 1, 2, 4, 8, 15, 30, 62 and 125 μg/ml) for 24 and 48 hours. Then MTT colorimetric assay was used to evaluate the cytotoxicity effect of ZEN and α-ZOL. Furthermore, morphological changes of treated and untreated cell lines were studied under an inverted microscope.

Results: The results obtained from the present study demonstrated that both ZEN and α-ZOL enhance the cell viability of MCF-7 especially at low doses (1 - 500 ng/ml) and at a high dose of 125μg/ml after 24 and 48 hours. However, this effect for α-ZOL was somewhat greater than that for ZEN. On the other hand, these estrogenic compounds did not have any effect on the cell viability of MDA-MB-468. No morphological change was observed in treated cells.

Conclusions: These results show that ZEN and α-ZOL enhance the rate of cell division in ER positive cells and therefore, exposure to this mycotoxin may increase the risk of breast cancer.

Keywords: Zearalenone, Breast Cancer, MCF-7, MDA-MB-468

1. Background

Cancer is a complex genetic disorder that is caused primarily by environmental agents. Human beings are constantly exposed to carcinogenic factors present in food, water, air, sunlight and chemicals (1). It is predicted that cancer-related morbidity and mortality, throughout the world, will rise alarmingly in the next few decades. Due to the anticipated population increase in the future years, we will see the incidence of cancer rise to 21.4 million by 2030, with nearly two thirds of all cancer diagnoses occurring in the low and middle-income countries (2).

Breast cancer encompasses 22.9% of all cancers in women and is more common in women than in men. The rate of incidence of breast cancer is ascending in all countries in the world especially in developing countries such as Iran (3, 4). In Iran, according to the National Center for Cancer Registration, breast cancer outbreak has increased significantly since 2001. In 2010, 23% of all cancers diagnosed in women were breast cancer cases. The incidence rate of breast cancer is estimated to be 22.09% in 100,000 women, with the age standardized incidence rate estimated as 28.25% in 100,000 women (4, 5). The American Cancer Society estimates that the incidence of breast cancer is 1 in every 8 women in the United States and the associated death rate is 1 in 36 (6).

Most important risk factors of breast cancer are non-preventable such as age, family history and medical history. However, there are some risk factors that are preventable such as overweight, physical inactivity, endocrine disrupting chemicals (EDCs), alcohol consumption, etc. (7-9). Since the late 1990s there has been an increased awareness and concern throughout the scientific world and the general population regarding EDCs. EDCs are estrogen-mimetic agents that intervene with the production, release, transport, metabolism, binding action or elimination of steroid hormones that are produced in the body for the maintenance of homeostasis and regulation of developmental processes (10). Epidemiological

and animal studies suggest that the major risk factor for breast cancer is overexposure to estrogens supported by the increased risk associated with early menarche, nulliparity, delayed pregnancy and late age of menopause (11). Because estrogen is known to influence the incidence of breast cancer and ablation of estrogen action remains the preferred treatment for hormone sensitive breast tumors, the presence of estrogenic chemicals in the breast area could influence the incidence of breast cancer (12). In vitro and in vivo studies have shown that natural or synthetic estrogenic compounds such as diethylstilbestrol (DES), Bisphenol A (BPA), parabens, phytoestrogens and mycoestrogens can induce cell proliferation, hypertrophy of female secondary sex organs, and synthesis of cell specific proteins (7, 13, 14).

The mycoestrogen Zearalenone (ZEN) is a non-steroidal estrogenic component with estrogenic activity produced by a variety of Fusarium fungi, which are common contaminants of cereal crops such as maize, wheat, barley, corn and rice worldwide (7, 15). Furthermore, it has been shown that ZEN can be excreted into cow milk (16). Studies indicated that ZEN and its metabolites disorganize reproductive functions in farm and laboratory animals and interfere with cancer-related diseases in humans (15-17). Following oral administration, ZEN is immediately absorbed and metabolized in the liver to α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL). ZEA and its derivatives are detected in blood about 30 minutes after feeding bound to human globulins as reproductive hormones (18, 19). Studies in mice showed that ZEN is distributed to estrogen target tissues such as uterus, ovarian follicles and testes interstitial cells. Some radiolabels were also found in adipose tissues, indicating that storage in adipose tissue may take place (16).

2. Objectives

Therefore, the present study was designed to investigate potential endocrine disrupting effects of ZEN and α-ZOL in the estrogen receptor positive and negative cell lines of the breast cancer, MCF-7 and MDA-MB-468 cells. The results obtained in this study were compared with latest observations (20).

3. Materials and Methods

Zearalenone and α-Zearalenol were purchased from Sigma-Aldrich. The stock solutions of these compounds were prepared in absolute ethanol and stored at 2 - 8°C. Serial dilutions for all compounds were made in culture medium. Dulbecco's modified Eagle's medium (DMEM): Ham's F12, fetal bovine serum (FBS), L-glutamine, trypsin-EDTA, and penicillin-streptomycin were purchased from Gibco (Gibco, Scotland).

3.1. Cells and Culture Conditions

Breast cancer human cell lines, MCF-7 (estrogen receptor-positive, ER+) and MDA-MB-468 (estrogen receptor-negative, ER −) were purchased from Iranian Biological Resource Center (Tehran, Iran). The cells were cultured in DMEM : Hams F12 (1:1) supplemented with 10% FBS, 2mM L-glutamine, and 1X penicillin-streptomycin, at 37°C in a humid incubator containing 5% CO_2.

3.2. Assessment of Cell Viability by MTT Assay

MTT colorimetric assay was applied to evaluate the cell viability effect of Zearalenone and α-Zearalenol on breast cancer cell lines MCF-7 and MDA-MB-468. Briefly, cells were harvested by trypsin-EDTA (0.25%) and re-suspended in fresh medium and seeded in 96-well culture plates at 4000 cells/well. Plates were then incubated at 37°C in 5% CO_2 for 24 hours. The culture medium was replaced by fresh medium containing Zearalenone and/or α-Zearalenol (0.1 nM to 125 μM) and plates were incubated at 37°C and 5% CO_2 for 24 and 48 hours. Then, MTT assay was performed and the percentage of the cell viability was evaluated using the equation: (mean OD of treated cells/mean OD of control cells) × 100. In this study, untreated cultures containing DMEM: Hams F12 supplemented with 10% FBS, 2mM L-glutamine, and 1X penicillin-streptomycin were used as controls to ensure reliable results. Moreover, all experiments were carried out in quadruplicate.

3.3. Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) in SPSS software (version 18). The results were expressed as the mean ± SEM and the levels of $P < 0.05$ were considered significant.

4. Results

In this study, MTT assay was used to evaluate the effects of ZEN and α-ZOL on viability of cell lines. As shown in Figures 1 and 2, treatment of the ER+, MCF-7 cells with different concentrations of ZEN and α-ZOL after 24 and 48 hours resulted in a significant ($P < 0.05$) increase in the cell viability. Both the ZEN and α-ZOL increased the viability of cells especially at low doses (1, 30, 60,125, 250 and 500 nm/ml) and a high dose 125μm/ml; however, the ability to increase cell viability was somewhat greater in α-ZOL compared to that of ZEN.

Statistical analysis of the effects of ZEN and α-ZOL on MDA-MB-468 demonstrated that these estrogenic compounds did not have any effect on cellular viability with all used concentrations after 24 and 48 hours (Figures 3 and 4).

4.1. Cell Morphology

Morphological analysis of treated cells in comparison to untreated cells by a microscope showed that ZEN and α-ZOL did not have any effect on the morphology of the cells after 24 and 48 hours.

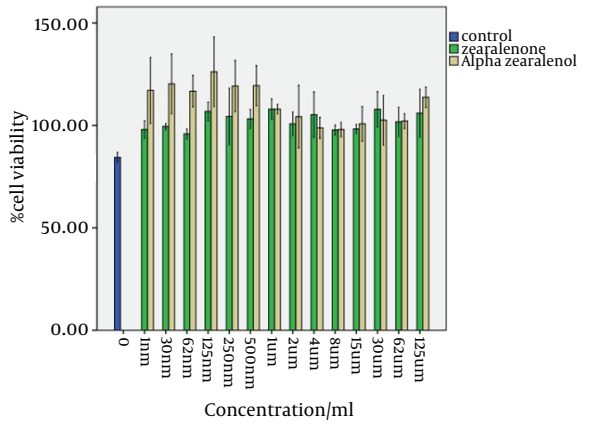

Figure 1. Effect of ZEN and α-ZOL on the Growth of MCF-7 Cells After 24 Hours Result Are Expressed as Percentage of the Control Without Treatment of ZEN and α-ZOL $P < 0.05$ Significant With Control

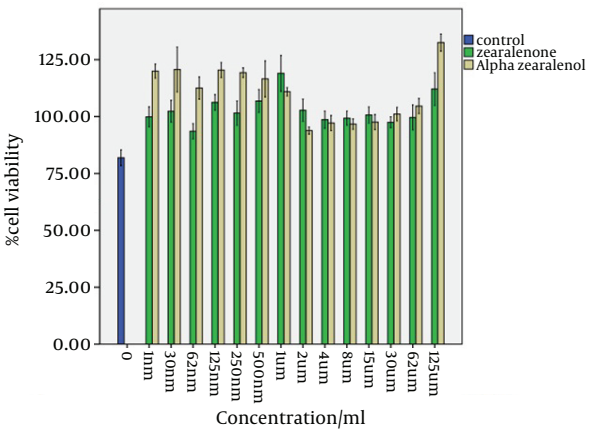

Figure 2. Effect of ZEN and α-ZOL on the Growth of MCF-7 Cells after 48 Hours Result are Expressed as Percentage of the Control Without Treatment of ZEN and α-ZOL $P < 0.05$ Significant With Control

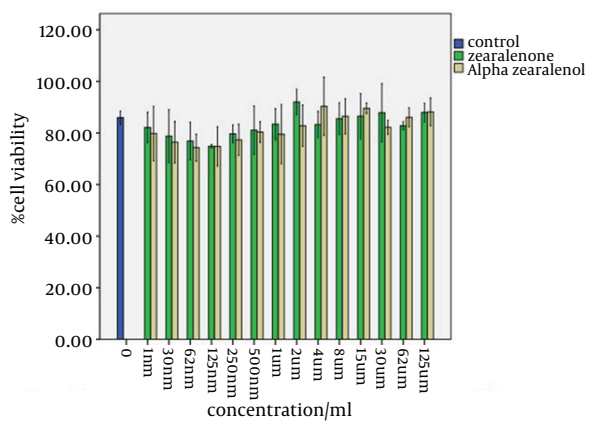

Figure 3. Effect of ZEN and α-ZOL on the Growth of MDA-MB-468 Cells After 24 Hours Result Are Expressed as Percentage of the Control Without Treatment of ZEN and α-ZOL $P < 0.05$ Significant With Control

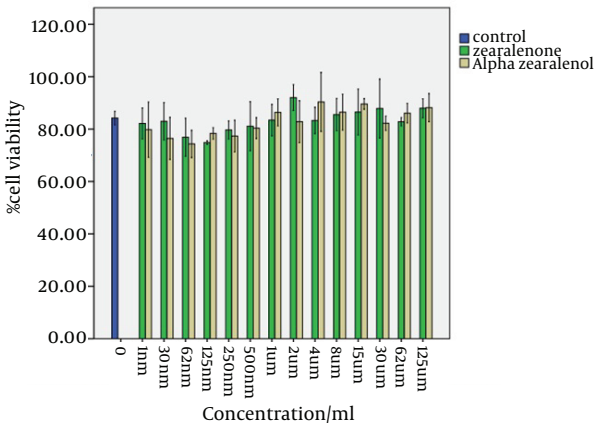

Figure 4. Effect of ZEN and α-ZOL on the Growth of MDA-MB-468 Cells After 48 Hours Result Are Expressed as Percentage of the Control Without Treatment of ZEN and α-ZOL $P < 0.05$ Significant With Control

5. Discussion

This study was designed to investigate the effects of ZEN and α-ZOL on cell viability of the breast cancer cell lines, MCF-7 and MDA-MB-468. Results indicated that ZEN and α-ZOL enhance the rate of cell viability in ER positive cells (MCF-7), but they did not have any effect on ER negative cells (MDA-MB-468). Therefore, it may be concluded that estrogen receptors may have an important role in cellular response to ZEN and α-ZOL and thus cell viability when cells are treated with these estrogenic compounds.

Several studies have shown that the molecular mechanisms involved in estrogen and estrogen mimetic compounds induce carcinogenicity. It is reported that, ER is an important prognostic factor in breast cancer cells (21-25). According to the molecular modeling calculations, ZEN and α-ZOL are able to occupy the active sites of the receptors in a rather similar way as 17β-estradiol (E2) does, because phenolic ring of these estrogenic compounds occupies the same region as the A-ring of E_2 (26). The results indicated that ZEN has strong estrogenic potentiality and activated both the AF-1 and AF-2 domains of ERs. These results also show that this mycoestrogen induced the rapid action-mediated response for ERα (27).

Bcl-2 oncoprotein acts as a cell death suppressor and is involved in apoptosis regulation in a number of systems, including mammary glands (28). The expression of Bcl-2 in ER positive breast tumors is higher than in ER negative ones. Herein, studies demonstrated that ZEN, like E2, increased the expression of Bcl-2 in ER positive breast cell lines such as MCF-7, but not in ER negative cells (29-31). In addition, cyclin D1 is a key cell cycle regulatory protein which controls cell cycle progression from G1 to S phase. The cyclin D1 gene is one of the most frequently amplified genes observed in human tumors and plays a fundamental role in the development of a subset of human cancers such as breast cancer (32, 33). A strong positive correlation between cyclin D1 and ER expression has also

been demonstrated in several studies (34-36). As such, strong cytoplasmic expression of cyclin D1 protein was observed only in MCF7 (with high expression of ER) and T47D cells (which moderately express ER) but not in ER-negative cells (MDA-MB-468) (25). Also, p53, a well-known tumor suppressor gene plays an important role in directing cells with DNA damage to apoptosis. The studies show that the p53 mRNA and protein levels were higher in MDA-MB-468 than in MCF-7. Breast tumors expressing a high level of p53 are more frequently ER-negative and associated with a higher proliferation rate and poorer survival (37, 38). ERα regulates the transcription of various genes as a transcription factor. The p53 has the ability to regulate ERα expression. It is possible that interaction between p53 and ER resulting in their reciprocal regulation, plays an important role in regulating normal breast epithelial cell proliferation and departure of this control may lead to breast cancer onset and progression (39). Thus, according to our observation and the results of other studies, it is clear that mycoestrogens including ZEN and α-ZOL and the presence of estrogen receptors on cells play an important role in the genesis and malignant progression of breast cancer.

It is important to note that ZEN produces DNA adducts and DNA ladders and leads to micronuclei and chromosomal aberrations. The signal transduction pathways by which mycotoxins mediate their toxic effects are believed to differ in a cell type dependent manner and depend on the exposed toxins (40). Moreover, other studies have shown that these mycoestrogens can play an important role in cellular propagation of estrogen- dependent cancers. ZEN, after ingestion by humans or animals, can affect cells directly, or be metabolized by organs and converted to α-ZOL, which is more potent than the primary compound (41). Epidemiological studies show that ZEN was found in blood plasma in high percentage of women with neoplasm of the mammary gland. A higher concentration of ZEN was noted in patients with benign tumors of the mammary glands (42). Therefore, we conclude that contamination of ZEN and its derivatives in food chains might contribute to the increasing incidence rates of breast cancer.

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