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Extended Abstract
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Pharma Sci-The Comparison Between the Cytotoxic Effect of Deinoxanthin and Mitomycin-C on MCF7 Cell Lines and the Changes on Bax and BCL2 Genes After Exposure- Minoo Iranshahi- Islamic Azad University

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Introduction

Deinococcus radiodurans is a well-known bacterium that is resistant to reactive oxygen species by exposure to ionizing radiation and oxidative stress. The antioxidant effect of this bacterium is highly related to carotenoids extracted from it Among the extracted carotenoids, deinoxanthin has been shown to have a very strong protective and restorative effect on DNA. The structure of this chemical has several conjugated dual bonds and also a hydroxyl group at position 1c. The superior antioxidant effect of deinoxanthin can be correlated with the unique chemical structure mentioned . Cancer can be considered as an imbalance between cell growth and death. Reduced cell death is due to the excessive expression of anti-apoptotic genes and inhibition of the expression of apoptosis genes. *BAX* and *BCL2* have been identified specifically as important proteins in the apoptosis, whose unbalanced expression, along with the presence of angiogenic agents, will exacerbate the decrementation of homeostasis in the body In addition, damage to the *BAX* and *BCL2* gene and the excessive expression of the anti-apoptotic genes, including *BCL2* have been observed in various cancers including melanoma, breast, prostate, and CLL, as well as in resistance to cancer treatment. In many studies, to eliminate cancer cells selectively, the use of strong antioxidants along with common chemotherapeutic agents have been proposed Also, in previous studies, induction of apoptosis of cancer cells by deinoxanthin was investigated and apoptotic and viability effects, morphological changes, and fragmentation of DNA were measured by oxidizing agents The results of studies showed that deinoxanthin has decreased the level of activity promoting enzyme Caspase 3 along with the expression of *BCL2*, a known gene against apoptosis in cancer cells and it increased the expression of *BAX* gene expression, the apoptotic gene The aim of the present study was to investigate the possibility of apoptotic properties of mitomycin-c and deinoxanthin. Due to the very different factors involved in the apoptosis, two genes from *BCL2* family have been considered as variables. The cytotoxic effect of mitomycin-c and deinoxanthin was evaluated on MCF7 breast cancer cell lines.

Materials and Methods

Materials and equipment

Blue, yellow and crystal tips were purchased from QC LAB (). Samplers were obtained from Nichipet EX II. 96 well plates were purchased from SPL (). Falcon (15 and 50 ml) and flask (25T) were obtained from JetBioFil (China). Plates, microtubes, vials, Neubauer chambers, and centrifuge tubes (5 ml) were all purchased from Biologix (). MTT assay kit was obtained from Biosera (France). Deinoxanthin was purchased from Chemistry & Chemical Engineering Research Center of Iran (Tehran, Iran). MCF7 (HUMAN BREAST ADENOCARCINOMA (IRBC CODE: C10682) cell lines were obtained from the Iranian Biological Resource Center (Tehran, Iran). Fetal bovine serum (FBS), DMSO, DMEM, and RPMI culture were obtained from Bio-Idea (Tehran, Iran). PBS (Phosphate buffered

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saline) and EDTA-Trypsin were purchased from Biosera (France). Mitomycin- C, penicillin and streptomycin were purchased from Sigma-Aldrich (Darmstadt, Germany). DEPC Treated Water and Rnx_Plus were acquired from Cinnagen (Tehran, Iran). Laminar flow cabinet (by KimyaGen, Tehran, Iran), Incubator (SCI FINETECH, Seoul, Korea), centrifuge (CENTURION, West Sussex, United Kingdom), Invert microscope made by Sunny (Japan). Cooling system, ELISA reader, Real-Time PCR by Bioneer, autoclave, and nanodrop device were used in this study.

Methods

Determination and preparation of deinoxanthin

The derived deinoxanthin characteristics have been compared to cytotoxic effects with pre-registered data for this compound. There are various techniques to examine the nature of this substance. One of the most commonly used techniques is UV spectroscopy and absorption spectroscopy of deinoxanthin. In this study, *Deinococcus radiodurans* culture was purchased from Chemistry & Chemical Engineering Research Center. Then, in the laboratory, it was determined by spectrophotometry technique and according to the reference; the presence of deinoxanthin was confirmed. Accordingly, the peak observed in the absorption spectrum of 220 nm proved the presence of deinoxanthin. After preparation of deinoxanthin in 15 ml Falcon, concentrations of 0.5, 1, 5, 10, 20, 30, 40 and 50 µg/ml were prepared by dilution with deionized water.

Preparation of different concentrations of mitomycin-C

A complete powder vial of mitomycin-c (2 mg) is completely dissolved in 4 ml water (concentration of 500 µg/ml). To measure the cytotoxic effect of mitomycin-C and compare it with deinoxanthin, 0.5, 1, 2, 5, 10 and 20 µg /ml concentrations of the prepared solution were prepared.

Preparation of DMEM medium

Based on the specification of the MCF7 texture obtained from Iranian Biological Resource Center, the appropriate environment for this category is DMEM, which contains essential amino acids, vitamins, and salts. To make this nutrient, 900 ml of deionized water was added into a 1 L Erlenmeyer flask and a magnet was placed in it. Then it was placed on a magnetic stirrer, and 37.16 g DMEM powder and 317 g sodium carbonate powder were weighed and added to the container as a buffer. After the solution is made, pH was adjusted in the range of 7.2-7.4 and it was sterilized with a 0.26-micron filter underneath the laminar flow cabinet. To prevent bacterial growth in this nutrient medium, 7.5 ml of penicillin-streptomycin antibiotic was added to the solution. Then 100 ml deionized water was added to the culture medium to reach a final volume of 1000 ml. In the end, underneath the laminar flow cabinet, the culture medium was filtered and transported in two 500 ml glasses and transferred to the refrigerator for storage. The stability time of the culture medium made is 2 weeks in the refrigerator.

Cell culture

DMEM medium was prepared with 10% bovine serum and penicillin-streptomycin 1% as a complete culture medium for cells. Some cells produce a lot of endogenous carbon dioxide. Every 24 to 48 hours, the cell culture medium is replaced. After covering the flask floor with bone marrow cells and achieving confluency of 70% - 87% in the initial culture, the was done accordingly: 1. culture medium in each flask was emptied, 2. the cell surface was washed with about 2 milliliter phosphate buffered saline PBS,

3.1 ml of trypsin-EDTA enzyme was added to 2 cm flask, 3. the flask was transferred to the incubator to increase the enzyme function for 3 minutes, 4. The flask was tapped a little too easily separate the cells from the flask floor, 5. trypsin effect was neutralized by adding a culture medium containing 17% FBS to 7.5 ml flask and pipetting and creating cell suspension, 6. The suspension was transferred to 15 ml Falcone and centrifuged at 1500 rpm at 25°C for 1.37 minutes. The supernatant was removed [7] cell culture medium containing 17% FBS was added to 1 ml of the precipitate. After pipetting and creating cell suspension, it was transferred to two of three new flasks in terms of cell density, 8.4 ml cell culture medium containing 17% FBS was added to each flask and the new flasks were transferred into the incubator, 9. Trypan blue was added to the suspension and the cells were counted before transferring the flasks to the incubator.

Freezing the cells

To freeze, the supernatant in the flask must be removed and the cells must be washed with PBS. The cells were then incubated for a few minutes at 37°C and purified by pipetting. After preparing the cell suspension, cell counting was done and the viability of the cells was determined. Based on the count, the number of required cryovials was determined and the cell characteristics, including the name of the cell line, the number of cells, the passage number, and the date of freezing, were inserted onto the cryovials. Then, the cry-vials were placed in ice and with the aid of the microtiter; the medium containing DMSO, FBS, DMEM was prepared and added to the cryovials. The cryovials were transferred to a freezer-20°C for 1-2 hours. It was then frozen at 80°C for 24 hours and eventually transferred to a nitrogen tank (196°C).

Defreezing the cells

To culture the desired frozen cell line, the cryovial was removed from the nitrogen tank and proceeded with caution and speed of action: 10 ml of culture medium containing 20% bovine serum and 80% DMEM with the antibiotic mixture was added to the flask. Then, in the cryovial was opened to release the nitrogen gas. Then it was placed on bain Marie and it was melted with rotational movements. Then, it was transferred into the flask. In the end, the flask incubated at 37°C containing 0.05% carbon dioxide.

Counting cells

The cell suspension was prepared in a volume of 1 ml and pipetted 20 µl of trypan blue 25% and the same volume of cell suspension was added in a 96-well plate pit. 10 microlitres of the mixture were placed on a Neubauer chamber.

Cell treatment with mitomycin C and deinoxanthin

After counting an MCF7 cell in a 1 ml volume, three columns of 96 well plates were assigned to MCF7 cells with 3,000 cells per well and two wells from the fourth column to the negative control. After 24 hr incubation period, deinoxanthin and mitomycin C solution were added to row 1 and 2 and one row was treated with both materials. A well without treatment was considered as a negative control. Then the color of cells was changed at MTT assay and was analyzed using ELIZA reader.

MTT assay

5 ml of RPMI1640 was added to a powder vial along with 12 ml of MTT solution and it was vortexed and the suspended particles were removed by centrifugation and filtration. Then, the clear solution was equally divided into 5 vials. Each vial is sufficient to be tested on 100 wells. The vial was stable at 4°C for 4 weeks. Other prepared vials for subsequent testing were stored

at -20 ° C for 12 months. After preparing the cells, 10 µl of 12 mM MTT solution was to each well and considered a well as a negative control. Then, 10 microliters of the stock solution were added to a non-cell culture medium. The plate was then transferred to a 37-degree incubator for 4 hr. After incubation, the plate was removed from the incubator and 50 µL of isopropanol solution was added to each well and it was placed in an incubator for 10 minutes. Then, the solution in each well was transferred to an ELISA reader for analysis at 570 nm.

Real-time PCR

cDNA molecules were denaturated at 95°C for 4 minutes. then, the temperature was maintained at 94°C for 30 seconds until the denaturation phase is completed, and then for 30 seconds, the temperature is reduced to 57°C until the DNA strands are paired and within 30 seconds Next, the temperature increased to 72 degrees to allow the DNA strand to elongate. The existence of different temperature steps provides for the propagation of 40 cycles.

Conclusion

Depending on the results of the study, it was found that deinoxanthin has a cytotoxic nature that significantly decreases the viability of MCF7 cells, which reduces the viability rate completely dependent on the dose and duration of exposure so that In doses higher than 30 µg/ml, viability was reduced to less than 20% during exposure at different times. Mitomycin-C at all concentrations has led to a decrease in cell viability and in doses of 10 and 20 µg/ml, the observed effect of using both substances together and mitomycin alone is similar. Thus, it can be seen that the cytotoxicity of both substances depends on the dose and duration of exposure. In examining the expressed genes, the *BAX* gene was induced by both materials but, contrary to the expectation, *BCL2* has not decreased. The results of the expression of *BCL2* and *BAX* in mitomycin indicate that the induction of apoptosis of mitotic-c is more effective than deinoxanthin and the use of Combination of it with deinoxanthin reduces the severity of induction of apoptosis by this gene, and this is important because it can create a new way to treat malignancy and reduce the incidence of complications due to the high and selective cytotoxicity of chemotherapy drugs.

References

1. Tian B, et al. Carotenoid biosynthesis in extremophilic Deinococcus–Thermus bacteria. Trends Microbiol. 2010;18:512-520.
2. Ji HF. Insight into the strong antioxidant activity of deinoxanthin, a unique carotenoid in *Deinococcus radiodurans*. Int. J. Mol. Sci. 2010;11:4506-4510.
3. Xu Z, et al. Identification and functional analysis of a phytoene desaturase gene from the extremely radioresistant bacterium *Deinococcus radiodurans*. Microbiology.2007;153:1642-1652.
4. Yang QF, et al. Expression of Bcl-2 but not *Bax* or p53 correlates with *in vitro* resistance to a series of anticancer drugs in breast carcinoma. Breast Cancer Res. Treat. 2000;61:211-216.
5. Verweij J, et al. Mitomycin C. Anticancer. Drugs.1990;1:5-14.
6. Kerr JFR, et al. Apoptosis: A Basic Biological Phenomenon with Wideranging Implications in Tissue Kinetics. Br. J. Cancer 1972;26:239-257.

7. Rzepczynska IJ, et al. Antioxidants Induce Apoptosis of Rat Ovarian Theca-Interstitial Cells1. Biol. Reprod. 2011;84:162–166.

8. Lowe SW, et al. Apoptosis in cancer. Carcinogenesis. 2000;21:485-495.

9. Dimitrakakis C, et al. Molecular markers in breast cancer: Can we use c-erbB-2, p53, bcl-2 and *bax* gene expression as prognostic factors? The Breast.2002;11:279-285.

10. Emi M, et al. Targeted therapy against Bcl-2-related proteins in breast cancer cells. Breast Cancer Res. 2005;7:42-49.

11. Antioxidant and Anticancer Activities of Methanol Extract of Melochia corchorifolia L. Int. J. Sci. Res.2017;6:1310-1316.

12. Lamson DW, et al. Antioxidants Ups and Downs. Sci. News.2000;157:147-150.

13. Cheng J, et al. Antioxidative and Hepatoprotective Activities of Deinoxanthin-Rich Extract from *Deinococcus radiodurans* R1 against Carbon Tetrachloride-Induced Liver Injury in Mice. Trop. J. Pharm. Res.2014;13:581-584.

Biography

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