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Evaluation of Cytotoxic Properties of All-Trans-Retinol on Breast Cancer and Prostate Cancer Cells

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ABSTRACT

Vitamin A and its derivatives (known as retinoids) play a crucial role in vision, inhibition of cell proliferation, cell division and differentiation and Fetal development. The active form of vitamin A is retinol, also known as vitamin A1 is found in animal products as preformed vitamin A. All-trans-retinol (vitamin A) has been studied as antioxidant in *in vitro* but limited data is available on cytotoxic effects of retinol on cancer cell lines. In this paper, the cytotoxic effects of retinol on breast cancer cells (MDA-MB-231) and prostate cancer cells (PC-3) were studied. Our experiments showed that maximum cytotoxicity was achieved at 48 hours and at 100µM concentration of all-trans-retinol on both the cell lines. The results have significant implications for understanding anti-cancer effects of all-trans-retinol on breast and prostate cancer cell lines.

Keywords: Vitamin A (all-trans-retinol), Cytotoxicity assays, MTT assay, Prostate cancer cells and Breast cancer cells.

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INTRODUCTION

Vitamin A and their derivatives are collectively known as retinoids, which include retinol (vitamin A alcohol), retinal (vitamin A aldehyde known as retinaldehyde) and retinoic acid (vitamin A acid form) [1]. Retinoids are involved in several biological processes such as vision, immune system function, embryonic development, reproduction, cell division and differentiation, skin and bone growth, hematopoiesis and gene transcription. Vitamin A can be obtained through diet either from animal sources or plant sources [2,3,4,5]. Epidemiological studies indicate that a low intake of vitamin A is associated with an increased risk of cancer. *In vitro* studies on cancer cells show that exposure to retinoids results in the inhibition of growth, by blocking the cell cycle or by inducing apoptosis. With respect to the clinical efficacy of retinoids some positive effects have been observed in early stage cancer .Recent studies have shed light on how retinoids inhibit the cancer growth in preneoplastic and neoplastic cells [6]. Structural details of vitamin A are given in Figure 1.

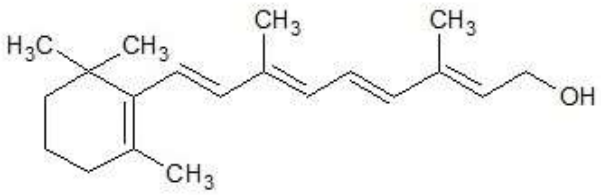

Figure 1: Structure of Retinol (Vitamin A)

IUPAC Name: (2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraen-1-ol

Among Vitamin A and its derivatives, all-trans-retinoic acid, and retinal have been widely studied for their cytotoxic effects [7,8]. In this paper, the cytotoxic effects of retinol on prostate cancer cells (PC-3 cell line), breast cancer cells (MDA-MB-231 cell lime) were studied, which will have implications on the applications of all-trans-retinol as one of the chemotherapeutic agents.

MATERIALS AND METHOD

Cell culture Media and Reagents

DMEM (Dulbecco's Modified Eagle Medium), MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) FBS (Fetal Bovine serum), DMSO (Dimethyl Sulphoxide), 96 well plate, Inverted Microscope, Micropipettes, Micro tips, Ficoll Histopaque 1077, Trypsin-EDTA solution 1X w/ 0.025% Trypsin and 0.01% EDTA in Dulbecco's Phosphate Buffered Saline sterile filtered all-trans-retinol procured from Acros Organics, Belgium.

Cell lines and cell culture

PC-3 (Prostate cancer) and MDA-MB-231 (Metastatic Breast Cancer) Cell lines was obtained from NCCS (National centre for cell science), Pune, India. The PC-3 and MDA-MB -231 cells were cultured in DMEM supplemented with 10% FBS and antibiotics. The cell lines were maintained at 37°C in a 5% CO_2 incubator.

Culture of mononuclear cells (MNCs) from peripheral blood

The protocol for the collection of the peripheral blood from healthy volunteers was approved by Institutional Ethics Committee of GITAM University. 5ml peripheral blood was collected from healthy volunteers in heparinized blood collection tubes after taking consent form the volunteers. Mononuclear cells from the whole blood were separated using density gradient centrifugation. The sample was carefully added to the layer of the Ficoll Histopaque 1077 in 15ml tubes. The tubes were centrifuged at 7000 rpm for 30 min without brake. The buffy coat was carefully aspirated and the mononuclear dense ring was collected in fresh sterile 15ml centrifuge tubes. The collected cells were washed two times using 10ml DMEM. The cell pellet was suspended DMEM enriched with 10% fetal bovine serum and antibiotics. The cells were cultured in 96-well plates and maintained in 5% CO_2 incubator at 37^0C [9].

Drug treatment and analysis of cell viability / cytotoxicity using MTT assay

Mononuclear cells were obtained as mentioned previously and cultured in DMEM serum containing medium. The trypsinised cancer cells from T-25 flask were incubated in a 96 well plate and allowed to adhere to the wells overnight in CO_2 incubator. Every time 5000 cells per well were taken. Cell lines were treated with different concentrations of retinol (1,10,100 µM solutions). Retinol was treated at various time intervals (24, 48 and 72 hours) and 20µl of MTT (5mg/ml in PBS) was added into each well and incubated in a CO_2 incubator until purple precipitate was visible. Then the supernatant was discarded and 200 µl of DMSO was added to each well to dissolve formazan crystals. The absorbance was read at a wavelength of 492nm on microplate reader [10,11].

The following formula was used to calculate cell viability using microplate reader at 492 nm.

$$\% \text{ cell viability } = \frac{\text{Test OD}}{\text{Control OD}} \times 100$$

$$\% \text{ Cytotoxicity} = 100 - \% \text{ Cell viability}$$

RESULTS AND DISCUSSION

The MDA-MB-231, PC-3 and mononuclear cells were treated with 1,10 and 100 micromolar concentrations of retinol. Table 1 has shown that cytotoxic effects of all-trans-retimol at 1 micro molar concentrations at 24, 48 and 72 hours on the above mentioned cell types. Table 2 indicates

that cytotoxic effects of all-trans-retinol at 10 micro molar concentrations at 24, 48 and 72 hours on the above mentioned cell types. Table 3 indicates that cytotoxic effects of all-trans-retinol at 100 micro molar concentrations at 24, 48 and 72 hours on the above mentioned cell types. All the tables have shown a dose – dependent increase in percent of cytotoxicity towards MDA-MB-231 and PC-3 cells with cytoxicity. It was found that at a concentration of 100 micromolar and at 48 hours of treatment the cell death was much higher in the metastatic breast cancer cell lines and prostate cancer cell lines than the mononuclear cells. This is certainly ideal as this indicates all-trans-retinol can probably be safely injested into the body without causing harm to healthy cells. From the above experiments it was understood that retinol interfere with cell replication process hence halting the cell cycle. The cytotoxicity was maximum at 100 micromolar concentration of retinol on MDA-MB231 and PC-3 cells.

Table 1: % of Cytotoxicity effects of 1 µM retinol on MDA- MB 231, PC-3 and MNCs

% of Cytotoxicity effects of 1µM Retinol on MDA-MB 231, PC-3 and mononuclear cells			
Time in Hrs	**% of Cytotoxicity on MDA-MB 231 cells**	**% of Cytotoxicity on PC-3 cells**	**% of Cytotoxicity on MNCs**
24	67.49±0.04	15.21±0.06	29.48±0.03
48	81.43±0.13	55.135±0.14	54.28±0.03
72	60.83±0.52	5.52±0.29	32.37±0.01

Table 2. % of Cytotoxicity effects of 10µM retinol on MDA-MB 231, PC-3 and MNCs

% of Cytotoxicity effects of 10µM Retinol on MDA-MB 231, PC-3 and mononuclear cells			
Time in Hrs	**% of Cytotoxicity on MDA-MB 231 cells**	**% of Cytotoxicity on PC-3 cells**	**% of Cytotoxicity on MNCs**
24	67.81±0.07	33.99±0.08	3.14±0.12
48	84.05±0.15	62.09±0.14	50.95±0.05
72	58.4±0.47	32.35±0.45	37.03±0.04

Table 3: % of Cytotoxicity effects of 100 µM retinol on MDA-MB-231, PC-3 and MNCs

% of Cytotoxicity effects of 100µM Retinol on MDA-MB 231, PC-3 and mononuclear cells			
Time in Hrs	**% of Cytotoxicity on MDA-MB 231 cells**	**% of Cytotoxicity on PC-3 cells**	**% of Cytotoxicity on MNCs**
24	63.0±0.14	69.73±0.06	36.06±0.02
48	80.08±0.14	69±0.10	55.62±0.04
72	58.81±0.53	57±0.35	43.56±0.02

CONCLUSION

MDA-MB231 (breast cancer) and PC-3 (prostate cancer) cell lines were treated with retinol in different concentrations (1,10 and 100 micromolars), at different time periods of incubation (24, 48 and 72 hours). The mononuclear cells isolated from healthy human volunteers peripheral blood were used as control for the above experiment. From these experiments it was estimated that 100 micromolar concentration was optimal to obtain the maximum cytotoxicity in cancer cells. This concentration did not affect the cell viability of the mononuclear cells. The optimal time of incubation was measured to be 48 hours.

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