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The effect of hormonal contraceptives on MDA breast cancer cell line

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Abstract

Breast cancer is one of the most prevalent malignancies worldwide, with hormone-responsive breast cancer being a significant subset of cases. Estradiol plays a crucial role in the proliferation and progression of hormone-sensitive breast cancer cells. To investigate the role of hormonal contraceptives, which commonly contain synthetic estradiol and progesterone in breast cancer development and progression. Treat MDA breast cancer cell line with Hormonal contraceptives (estradiol and progesterone and mix of both) with different concentration, replicates was 3 technical replication in one experiments was performed. The result was the estradiols have high effect on progression of cancer and increase the cell viability. Hormonal contraceptives increase the breast cancer cell and cell viability especially that have high concentration of estradiol.

Keywords: breast cancer, estradiol, Synthetic hormonal contraceptives, MDA cell line, progesterone.

Introduction

Breast cancer stands as a formidable global health challenge, characterized by its diverse molecular subtypes and complex etiology. Among the factors implicated in breast cancer development and progression, hormonal influences, particularly the actions of estrogens and progestogens, have been extensively documented[1]. Estrogens, specifically estradiol, are known to induce the proliferation of hormone-sensitive breast cancer cells, addition to contributing to tumor growth and progression. Consequently, the widespread use of hormonal contraceptives, which introduce exogenous synthetic estrogens and progestogens into the human physiological system, has raised the questions regarding their ability impact on breast cancer risk[2]. While these contraceptives offer significant benefits in family planning and management of gynecological conditions, the need to thoroughly investigate their influence on breast cancer at a cellular level remains paramount. To understand this, in vitro models, such as the MDA cell line, provide a valuable platform for studying the direct effects of hormonal compounds on cancer cell

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behavior[3]. The MDA cell line, derived from a metastatic site and representing the aggressive triple-negative breast cancer (TNBC) subtype, allows researchers to isolate and examine the cellular mechanisms through which hormonal contraceptives may effect proliferation, invasion, and other critical cancer-related processes[3]. MDA cell lines, particularly MDA-MB-231, are often used as models for triple-negative breast cancer (TNBC) due to their lack of classical estrogen receptor alpha (ER alpha), progesterone receptor (PR), and HER2 amplification.

However, estradiol and progesterone can still impact these cells through non-classical, rapid signaling pathways involving GPER1, ER beta, and membrane-bound receptors[4]. **G protein-coupled estrogen receptor 1 (GPER1)** is a seven-transmembrane receptor primarily localized to the plasma membrane and endoplasmic reticulum. Upon binding to estradiol, GPER1 activates rapid signaling cascades, including the activation of G proteins, adenylyl cyclase, protein kinase A (PKA), and the transactivation of growth factor receptors like EGFR. This leads to restore their ability to stimulate adenylyl cyclase and attenuate EGF-induced activation of Erk-1/-2 by estrogen[5]. GPER1 activation can also lead to increased proliferation or inhibition of apoptosis depending on the cellular context and other signaling molecules involved[6]. **Membrane-bound Estrogen Receptor Alpha 36 (ER alpha36)** it mediates rapid estrogenic signaling by interacting with various signaling molecules, including Src, MAPK/ERK, and PI3K/Akt pathways, often leading to transactivation of growth factor receptors[7]. Estradiol, through ER alpha36, has been implicated in promoting aspects of cancer progression like cell growth, inhibition of apoptosis, and metastasis, potentially through pathways like phospholipase C (PLC) and protein kinase C (PKC)[8]. This research endeavors to elucidate the specific effects of hormonal contraceptive components on the proliferation of MDA cells, aiming to contribute to a deeper understanding of the pathways involved and to inform evidence-based clinical guidelines concerning hormonal contraceptive use and breast cancer risk assessment.

Materials and Methods

MDA cell line

The MDA cell line is a well-established and widely used tool in breast cancer research.It is a human breast cancer cell line, was established from a pleural effusion (fluid buildup in the lungs) of a 51-year-old Caucasian female with metastatic mammary adenocarcinoma. It is a highly aggressive and invasive triple-negative breast cancer (TNBC) cell line, This means it lacks expression of estrogen receptors (ER), progesterone receptors (PR), and HER2 (human epidermal growth factor receptor 2) amplification[9] .

Cell Seeding

When reached confluent 70-80% monolayer, MDA cells were detached from culture flask by trypsinization, then 5 mL of complete growth RPMI-1640 medium were added to the flask and mixed gently to prepare cell suspension. Aliquot of 200 µL of cell suspension was transferred to each well in micro titration plate (each well contain approximately 1 x 10 cell/well). The plate was covered with a sterile adhesive film, gently shook for few min and incubated at 37 ºC, 5% CO2 for 24 hr[10].

MTT Cell Proliferation Assay

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The MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) is a reliable indicator of the cellular metabolic activity. The principle of MTT assay is based on the reduction of MTT, a yellow water-soluble tetrazolium dye, by the mitochondrial dehydrogenases of the viable cells, to violet-blue water-insoluble formazan[11]. 20 µL of MTT solution was added into each well and incubated at 37°C for 2 hours and 50 µL of DMSO was added into each well afterward. The absorbance was read at 570–630 nm by microplate Reader (Huma Reader, HS, Germany).

Statistical Analysis

The obtained results were analysed using SPSS 2018[12]. In order to display the descriptive analysis for the studied parameters, the error bar was used as a dependent variable. The effect of the hormonal contraceptives (estradiol and progesterone) and mix of both Complex on the MDA cell line was considered statistically significant if the p-value was less than 0.05.

Results

The MTT assay was used to evaluate the increase of cell viability that treated with Estradiol, progesterone and mix of both hormones as they are most commonly used as hormonal contraceptives (HC). The result by one way ANOVA shows the effect of Estradiol, Progesterone, and mix of both on MDA cell line viability, we see that estradiol has the most potent stimulatory effect on MDA cells at the highest concentration (500.00 µg/ml), the mean is 132.50, and at the lowest one (7.80 µg/ml), it drops to 101.77. Progesterone concentrations also show the mean at 500.00 µg/ml is 121.15. Mixed Concentrations for both hormones was 103.02 at 500.00 µg/ml.

Table (1): The effect of different concentration of Estradiol, Progesterone, and mix of both.

Conc.(µg/ml)	Estradiol Mean± SD (N)	Progesterone Mean± SD (N)	Mixed Mean± SD (N)
500.00	132.50 ±22.25 (3)	121.15 ±16.08 (3)	103.02± 4.52(3)
250.00	119.69 ± 21.71 (3)	110.10 ±18.72 (3)	99.90± 11.08 (3)
125.00	118.75 ± 8.71 (3)	108.02 ±14.55 (3)	90.94± 9.79 (3)
62.00	111.98 ± 15.25 (3)	103.02 ±11.75 (3)	90.94 ± 6.15 (3)
31.00	110.10 ± 7.09 (3)	97.92± 11.76 (3)	84.38 ± 7.60 (3)
15.00	109.38 ± 1.36 (3)	96.67± 5.16(3)	85.94± 5.49 (3)
7.80	101.77 ± 9.21 (3)	91.67 ± 1.41(3)	84.17 ± 4.77 (3)

The mean (in µg/ml) of Estradiol and Progesterone across various dosage levels, including their standard deviations (SD) and sample sizes (N).

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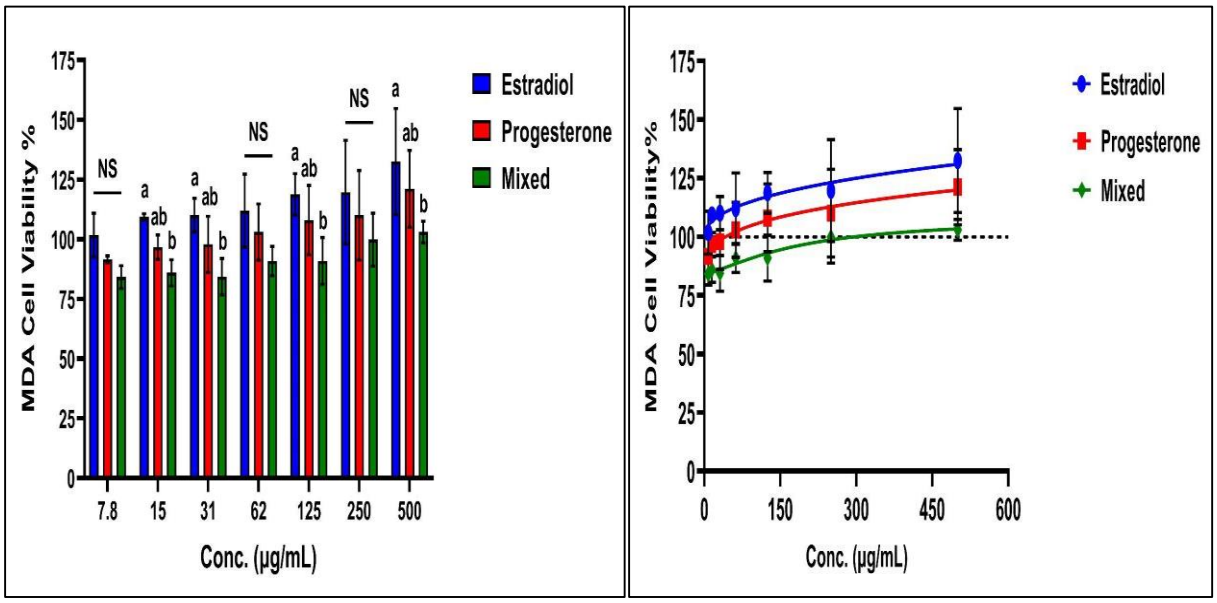

Figure (1): the effect of different concentration of Estradiol, Progesterone, and mix of both

Table (2): the effect of different concentration of Estradiol, Progesterone, and mix of both

Concentrations (µg/ml)	Tukey's multiple comparisons test	P Value
7.8	Estradiol vs. Progesterone	0.5509 (NS)
	Estradiol vs. Mixed	0.1733(NS)
	Progesterone vs. Mixed	0.7182(NS)
15.0	Estradiol vs. Progesterone	0.3928(NS)
	Estradiol vs. Mixed	0.0497 *
	Progesterone vs. Mixed	0.5112(NS)
31.0	Estradiol vs. Progesterone	0.4227(NS)
	Estradiol vs. Mixed	0.0284*
	Progesterone vs. Mixed	0.3474(NS)
62.0	Estradiol vs. Progesterone	0.6248(NS)
	Estradiol vs. Mixed	0.0858(NS)
	Progesterone vs. Mixed	0.4288(NS)
125.0	Estradiol vs. Progesterone	0.5112(NS)
	Estradiol vs. Mixed	0.0165*
	Progesterone vs. Mixed	0.1911(NS)
250.0	Estradiol vs. Progesterone	0.5844(NS)
	Estradiol vs. Mixed	0.1120(NS)
	Progesterone vs. Mixed	0.5442(NS)
500.0	Estradiol vs. Progesterone	0.4725(NS)
	Estradiol vs. Mixed	0.0105*
	Progesterone vs. Mixed	0.1568(NS)
*significant difference		

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Discussion

Our findings reveal a clear hierarchy in the ability of these hormones to influence the growth of MDA breast cancer cells, as measured by the MTT assay. Estradiol, the primary female sex hormone, emerged as the most potent stimulator of cell viability. This observation aligns with a growing body of evidence indicating estradiol's pivotal role in driving the proliferation of certain breast cancer subtypes[13]. Recent studies have consistently shown that estradiol can activate signaling pathways that promote cell division and survival in breast cancer cells [14]. While MDA cell lines, particularly MDA-MB-231, are often used as models for triple-negative breast cancer (TNBC) due to their lack of classical estrogen receptor alpha (ER alpha), progesterone receptor (PR), and HER2 amplification, estradiol can still impact these cells. This occurs through non-classical, rapid signaling pathways involving G protein-coupled estrogen receptor 1 (GPER1) and membrane-bound Estrogen Receptor Alpha 36 (ER alpha36). GPER1, upon binding to estradiol, activates rapid signaling cascades leading to increased proliferation or inhibition of apoptosis depending on the cellular context. Similarly, ER alpha36 mediates rapid estrogenic signaling by interacting with pathways like Src, MAPK/ERK, and PI3K/Akt, and estradiol via ER alpha36 has been implicated in promoting cancer progression aspects like cell growth and inhibition of apoptosis[15]. This is important, because this cell line is a triple negative breast cancer cell line, meaning it does not express the estrogen receptor. However, there are other pathways that estradiol can activate [16].

Progesterone, while also demonstrating a stimulatory effect, exhibited a less pronounced impact compared to estradiol. This is important, because progesterone can have multi effects on breast cancer cells, sometimes even showing inhibitory properties [17]. However, in our research we saw a stimulatory effect. The mix treatment with estradiol and progesterone show an intermediate effect, falling between the individual effects of the two hormones. This indicates a possible interaction or modification of their effects when used concurrently. Progesterone may be alleviating some effects of estradiol [18].

This observed trend highlights the intricacy of hormonal effects on breast cancer cell activity. It also underscores how various hormones, even those with similar activities, may have disparate impacts on cell survival. The observation that estrogen has the most significant stimulatory impact underscores the need of comprehending the mechanisms activated by estradiol in MDA cells [19]. Additional study is required to completely clarify the fundamental processes and any clinical ramifications of these results. Comprehending the impact of these hormones on breast cancer cell proliferation is essential for formulating focused treatments and enhancing risk evaluations about hormonal exposures.

Conclusions

Exposure to high concentration of estradiol can induce cell proliferation and cell viability in cancer tissue even the cells lack to estradiol receptor.

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

Sahar M. Ibrahim: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

All Authors: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

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