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The Effect of Hormonal Treatment on Cell Viability in F98 Cell Line

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ABSTRACT The aim of the present study is to investigate the effects of three different steroid hormones; 17 β estradiol, Diethylstilbestrol and progesterone on cell viability in F98 glioblastoma cells. F98 glioblastoma cells were treated with different concentrations of Progesterone (10, 20, 50, 100 µM), DES (2.5, 5, 10, 20, 50, 100 µM) and 17β estradiol (0.01, 0.1, 1, 10 µM)) for 24, 48 and 72 hours and MTT assay was applied to determine thecell viability. Progesterone inhibits glioblastoma cell growth in a dose and time dependent manner. Antiproliferative effect of 17 β estradiol was observed at low doses. Biphasic distribution was observed in decreasing cell viability in DES applications. These results suggest that Progesterone, 17β estradiol and DES can inhibit the proliferation of glioblastoma cells. However, further study is necessary to identify the pathways involved.

Keywords: *Diethylstilbestrol, Estrogen, Glioblastoma, Progesterone*

ÖZ

Hormon Uygulamalarının F98 Hücre Hattında Hücre Canlılığı Üzerine Etkisi

Bu çalışmada 17 β östradiol, Dietilsitilbesterol ve progesteron gibi steroid hormonların F98 glioblostomo hücre hatlarında hücre canlılığı üzerine etkilerinin araştırılması amaçlanmıştır. F98 glioblastoma hücrelerine farlı dozlarda Progesteron (10, 20, 50, 100 µM), DES (2.5, 5, 10, 20, 50, 100 µM) ve 17β estradiol (0.01, 0.1, 1, 10 µM) 24, 48 ve 72 saat süre ile uygulanmış ve hücre canlılığının belirlenmesi amacıyla MTT hücre canlılık testi uygulanmıştır. Progesteron glioblostoma hücrelerinin büyümesini doz ve zaman bağımlı olarak inhibe etmiştir. 17 β estradiol düşük dozlarda antiproliferatif etki göstermiştir. DES uygulamaları hücre canlılığı üzerinde iki yönlü etki göstermiştir. Elde edilen sonular Progesteron, 17β estradiol ve DES'in glioblostoma hücrelerinde hücre çoğalmasını inhibe ettiğini göstermektedir. Ancak bununla ilgili yolakların belirlenmesi için daha fazla çalışma yapılmasına ihtiyaç vardır.

Anahtar Kelimeler: *Dietilstilbestrol, Östrojen, Glioblastoma, Progesteron*

INTRODUCTION

Glioblastoma (GB) is a very common malignant primary brain tumor in adults. Though these tumors occur mostly in adults, no age is immune. It has a very poor prognosis. It was previously known as glioblastoma multiform. Surgical resection, followed by radiotherapy and chemotherapy is applied fortherapeutic purpose (Virgil et al.2018).

The incidence of glioblastoma in general increases with age. Men have higher incidence than woman showing estrogen may have protective effects in women. In glioma development endogenous estrogens could have beneficial effects which explain that men are approximately 1.5–2 times more likely to develop proportional GB. In experimental studies, glioblastomas transplanted female animals showed a slower growth rate than in male animals (Kabat et al. 2010). Steroid hormones are biosynthesized in the mitochondria. Cholesterol derivatives are used for the synthesis of steroid hormones (Miller and Bose2011) based on their biosynthesis, steroid hormones are classified as corticosteroids and sex steroids. Sex steroids (estrogen, progesterone, and androgen) are responsible for the development and maintenance of sexual characteristics in addition to general reproductive function. Besides their role in normal reproductive development, sex steroids may also play a role in the development and progression of several cancers (Marceau et al.2015). Estrogens are steroid hormones that exert important effects on the reproductive and gastrointestinal systems, mammary glands, skeletal and immune systems, and even the central nervous system (Tavares et al. 2016). Diethylstilbestrol (DES) is a synthetic form of the female hormone estrogen. DES also hasbeen used in the treatment of prostate cancer to reduce testicular androgen production secondary to inhibition of LH released from the pituitary gland (Ali Shah 2015).

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Progesterone participates in the regulation of several reproductive processes, including ovulation and sexual behavior. In synergism with estrogen, progesterone also influences neuronal excitability; learning and neoplastic proliferation of glial cells (Tavares et al. 2016). Steroid hormones play a key role in brain development and differentiation. Furthermore, ovarian steroid hormones are neuroprotective in a variety of neurologic disorders. These neuroprotective effects include improved myelination, decreased edema, inhibition of apoptosis and decreased inflammation (Tavares et al. 2016). Steroid hormones may also play a role in the development of brain tumors, because steroid hormone receptors are members of a superfamily of ligand-activated transcription factors that are potentially oncogenic (Kabat et al. 2010). In this study the effect of 17 β estradiol, Diethylstilbestrol (DES) and progesterone on glioblastoma cells were investigated.

MATERIALS and METHODS

F98 rat glioblastoma cells were cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum at 37°C in a 5% CO_2 environment. Progesterone, DES (a synthetic estrogen) and estrogen stocks were prepared in dimethylsulfoxide (DMSO) and further diluted in culture medium. The final concentration of DMSO used in dilution was kept at <2.5µl/ml. Cells were exposed to different concentrations of Progesterone (10, 20, 50, 100 µM), DES (2.5, 5, 10, 20, 50, 100 µM) and 17β estradiol (0.01, 0.1, 1, 10 µM)) for 24, 48 and 72 hours. Glioblastoma cell viability was assessed by MTT assay. The MTT assay is widely used to measure cell death/proliferation. In the reaction tetrazolium ring is cleavage to the pale yellow MTT into dark blue formazan crystals. Mitochondrial dehydrogenase enzyme in viable cells is responsible in this reaction. Formazan crystals impermeability to the cell membrane thusaccumulate within the cells and SDS (100µl) is added to solubilize these crystals. The intensity of blue formazan solution is directly proportional to the number of surviving cells. Concentrations were determined by photometric analysis. Briefly, 10 µl of MTT solution (5 mg/ml in phosphate buffered saline) was added per well and incubated at 37°C for 4 h until a purple precipitate was visible. SDS (100 µl) was added to solubilize the crystals and the absorbance was read at 570 nm.

Statistical Analysis

The experiment was replicated at least four times. The results were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's post hoc test to determine significant differences in all parameters among all groups. SPSS/PC computer program (IBM SPSS Statistics version 22.0) was used for statistical tests and differences with values of $P < 0.05$ were considered statistically significant.

RESULTS

Cell viability of rat F98 glioblastoma cells was measured at 24, 48 and 72 h after exposed todifferent concentrations of Progesterone (10, 20, 50, 100 µM), DES (2.5, 5, 10, 20, 50, 100 µM) and 17β estradiol (0.01, 0.1, 1, 10 µM)(Table 1, 2, 3). After 24-hour exposure, decreased cell viability in all doses of DES applicationswere observed. Decrease in all groups compare to control group was significant (p <0, 05). After 48 hours cell viability in 20 µM and 50 µM DES applied cell was the same level compare to control cells. But in other doses cell viability decreased compared to control group (p <0,05). After 72-hour exposure there was a reduction in cell viability in all groups compare to control group (p <0,05). Most distinctive reduction in cell viability/proliferation of F98 cells in72h application was in 100µM DES applied cells (Figure 1).These differential effects could be due to the activation of distinct signaling mechanisms by DES, depending on its concentration and time. DES, a synthetic form of the female hormone estrogen, had remarkable effect on cell viability in all doses after 24-hour exposure (Figure 1). 17 β estradiol estrogens showed antiproliferative effect on cell viability. Antiproliferative effect of 17 β estradiol was observed at all doses (0.01-1 µM) (p <0, 05) after 24 hours. There were no differences in cell viability at 10µg 17 β estradiol concentrations compare to control (Figure 2).Cell viability in rat F98 glioblastoma cells at 48 and 72 hours decreased in all doses of 17 β estradiol compare to control (p <0,05).

Table 1. The mean (± SD) cell viability (%) of F98 cells, 24h, 48h and, 72h exposure of different DES concentrations (2.5, 5, 10, 20, 50, 100 µM)

Groups	24 h	n	48 h	n	72 h	n
	% cell viability (± SD)		% cell viability (± SD)		% cell viability (± SD)	
Control	99.83 ± 5.46^{a}	6	100.00 ± 13.83^{a}	7	100.13 ± 11.18^{a}	8
DES (2.5 µM)	50.67 ±7.64^{b}	6	79.86 ± 13.11^{b}	7	67.00 ± 4.05^{c}	6
DES (5 µM)	40.45 ± 6.37^{bc}	6	78.00 ± 13.25^{b}	7	64.40 ± 5.64^{c}	5
DES (10 µM)	37.23 ± 4.67^{bc}	6	73.14 ± 5.43^{b}	7	66.40 ± 11.01^{c}	5
DES (20 µM)	46.23 ± 6.88^{bc}	6	99.25 ± 7.54^{a}	4	84.40 ± 4.56^{b}	5
DES (50 µM)	42.05 ± 7.18^{c}	6	100.88 ± 12.91^{a}	8	77,88 ± 6,62^{b}	8
DES (100 µM)	46.27 ± 13.06^{c}	6	54.33 ± 28.36^{c}	6	13,17 ± 5,74^{d}	6
P value	**<0,05**		**<0,05**		**<0,05**	

$^{a, b}$ Different superscripts within the same column demonstrate significant differences

Table 2. The mean (± SD) cell viability (%) of F98 cells 24h, 48h and, 72h exposure of different progesterone concentrations (10, 20, 50, 100 µM)

Groups	**24 h**	**n**	**48 h**	**n**	**72 h**	**n**
	% cell viability (± SD)		% cell viability (± SD)		% cell viability (± SD)	
Control	80.65 ± 40.40[a]	6	79.00 ± 3.91[a]	6	100.33 ± 14.14[a]	6
Progesterone (10 µM)	76.57 ± 12.38[a]	6	64.83 ± 21.74[ab]	6	60.17 ± 25.64[bc]	6
Progesterone (20 µM)	70.47 ± 36.10[a]	6	50.50 ± 31.13[ab]	6	54.60 ± 26.65[bc]	6
Progesterone (50 µM)	64.00 ± 2.86[a]	6	70.17 ± 3.49[ab]	6	69.83 ± 3.60[ab]	6
Progesterone (100 µM)	20.85 ± 11.02[b]	6	36.80 ± 18.03[b]	5	35.33 ± 16.53[c]	6
P value	**<0,05**		**<0,05**		**<0,05**	

[a,b] Different superscripts within the same column demonstrate significant differences

Table 3. The mean (± SD) cell viability (%) of F98 cells 24h, 48h and, 72h exposure of different 17 β estradiol concentrations (0.01, 0.1, 1, 10 µM)

Groups	**24 h**	**n**	**48 h**	**n**	**72 h**	**n**
	% cell viability (± SD)		% cell viability (± SD)		% cell viability (± SD)	
Control	99.83 ± 5.46[a]	6	100.00 ± 13.83[a]	7	100.17 ± 5.35[a]	6
17β estradiol (0.01 µM)	60.38 ± 7.41[b]	8	89.50 ± 26.56[ab]	4	68.71 ± 12.98[b]	7
17β estradiol (0.1 µM)	71.00 ± 24.51[b]	8	74.17 ± 10.91[b]	6	71.67 ± 6.35[b]	6
17β estradiol (1 µM)	61.00 ± 17.47[b]	7	72.71 ± 6.53[b]	7	70.33 ± 15.25[b]	6
17β estradiol (10 µM)	93.38 ± 17.00[a]	8	78.25 ± 5.85[b]	4	75.00 ± 6.44[b]	5
P value	**<0,05**		**<0,05**		**<0,05**	

[a,b] Different superscripts within the same column demonstrate significant differences

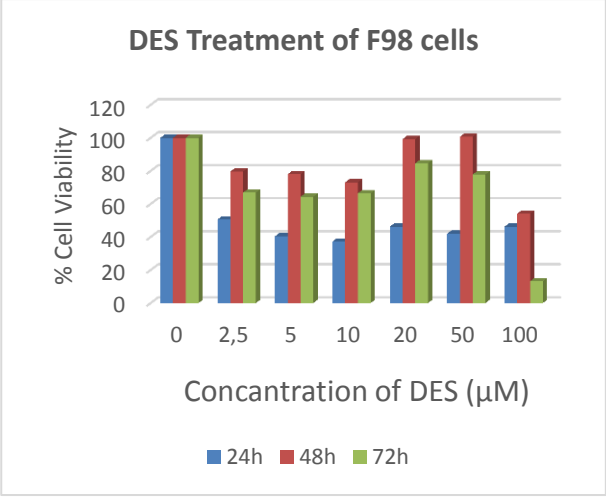

Figure 1. Cell viability assay. Graph of MTT assay showing the rate of viability of F98 cells 24h, 48h and, 72h exposure of different DES concentrations (2.5, 5, 10, 20, 50, 100 µM) along with controls.

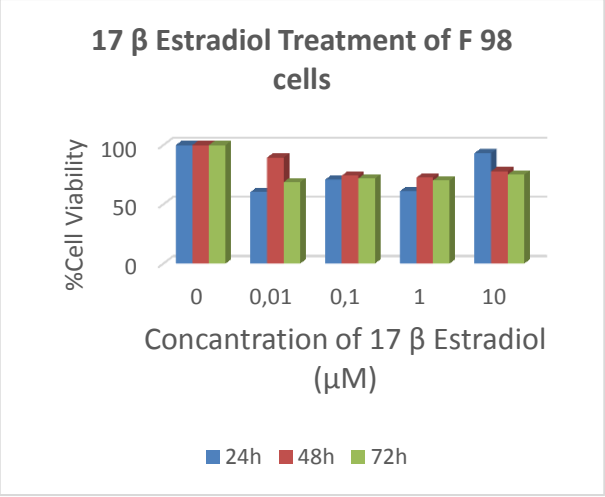

Figure 2. Cell viability assay. Graph of MTT assay showing the rate of viability of F98 cells 24h, 48h and, 72h exposure of different 17 β estradiol concentrations (0.01, 0.1, 1, 10 µM) along with controls.

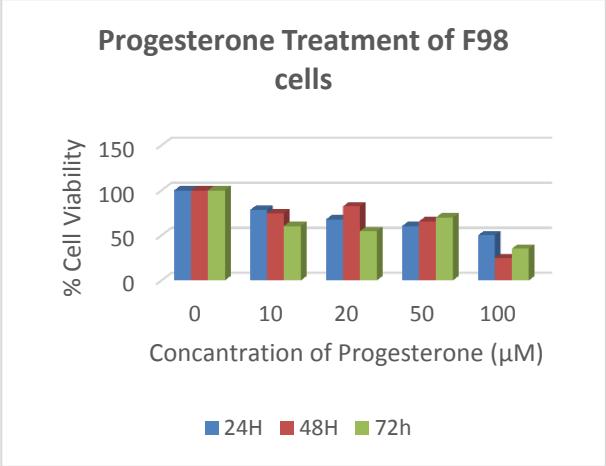

Figure 3. Cell viability assay. Graph of MTT assay showing the rate of viability of F98 cells 24h, 48h and, 72h exposure of different progesterone concentrations (10, 20, 50, 100 µM) along with controls.

DISCUSSION

In various functions related to the brain, including central and peripheral nervous system development, neurotransmitter systems regulation, and myelination, steroid hormones have a role in regulation. In addition to these function properties, they have also role in cognition, emotion, mood, sexual behavior and social behavior (Rossetti et al. 2016). Cholesterol or steroidal precursors imported from both neurons and glia are used de novo synthesis of steroid hormones. Locally synthesized hormones, neurosteroids, binds their receptorsto regulate several central nervous system functions. They also play a role in neurodegenerative disease and ageing mechanism (Rossetti et al. 2016). In the brain and other tissues, depending on the estrogen receptors (ERs) concentration and the expression estrogen may induce cell growth or cell death. According to Altiok et al. estradiol induces apoptosis and suppresses cell growth in a concentration and time-dependent manner in C6 glioma and T98G glioblastoma cells. SinceC6 glioma and T98G glioblastoma cells express ERs, estradiol may have effects glioblastoma cells because it regulates the ER-mediated transcription of genes involved in cell survival and death and activates of intracellular signaling pathways on neurons and glial cells (Altiok et al. 2011). According to Ho et al., the incidence of gliomas has increased in the last 21 years (Ho et al. 2014). Estrogens may influence the development and control of brain tumor growth by interacting with their receptors or activating potentially oncogenic mediators. Estrogens seem to have a protective effect on the development of gliomas because they occur more commonly in men than in women. In women, the incidence of gliomas increases during the postmenopausal period, when estrogen levels are low (Dueñas Jiménez et al. 2014; Kabat et al. 2010; Patel et al. 2012). In previous study, it was indicated that estradiol suppresses cell growth in C6 glioma and T98G glioblastoma cells (Altiok et al. 2011). In our study, similar result obtained in F98 cell line. But comparing the effect of diethylstilbestrol (DES), 17 β estradiol did not have remarkable effect on cell viability. This effect may be due to the fact that DES is estimated to be five times more potent than the naturally occurring estrogen, estradiol (Korach et al. 1978; IARC 2012). Hormonally inactive compounds (such as β-di-enestrol) or compounds that retain estrogenic activity (like DES-epoxide or quinone metabolites) are produced following to oral absorbsion and metabolism of DES (Korach et al. 1978; IARC 2012). *In vitro* studies indicate that progesterone promotes cell proliferation in astrocytomas, as well as the expression of genes that are important for tumor growth and dissemination, for example vascular endothelial growth factor (Dinget al. 2000). However, there are several studies in the literature confirming that progesterone has anti-proliferative and apoptotic effects on ovarian, breast, endometrial and colon tumors as well as gliomas (Atif et al. 2015; Cabrera-Muñoz et al. 2011; Tang et al. 2006). According to Atif et al., high doses of progesterone inhibit the growth of glioblastoma multiforme, both in vitro and in animal experiments. This effect was shown to mainly involve the inhibition of cellular proliferation and angiogenesis and the induction of apoptosis. They also found that progesterone improves Temozolomide (TMZ)'s (an anticancer agent) efficacy in glioblastoma cells and reducing its adverse effects it protects primary healthy cells (Atif et al. 2015).

CONCLUSION

These findings suggest those hormones such as progesterone, diethylstilbestrol (DES), and 17 β estradiol can reduce glioblastoma cells proliferation. There is need for further studies to clarify their way of action.

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