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

Burcu Menekşe BALKAN†, Görkem KISMALI‡, Soner CENGİZ§, Tevhide SELслуж

ABSTRACT

The aim of the present study is to investigate the effects of three different steroid hormones; 17β 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 the cell viability. Progesterone inhibits glioblastoma cell growth in a dose and time dependent manner. Antiproliferative effect of 17β 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

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 progression of several cancers (Marcceu et al2015). 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 has been 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).

Corresponding author: Burcu Menekşe BALKAN
Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Biochemistry, Burdur, Turkey, e-mail: buralcan railcom

† Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Biochemistry, Burdur, Turkey
‡ Ankara University Faculty of Veterinary Medicine, Department of Biochemistry, Ankara, Turkey
§ Kayseri University, Yeşilhisar Vocational Collage, Department of Animal Science, Kayseri, Turkey

Received: 16.09.2019 Accepted: 05.03.2020
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 neoptelic 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% CO₂ 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)(Table 1, 2, 3). After 24-hour exposure, decreased cell viability in all doses of DES applications were observed. Decrease in cell viability at all doses (0.01, 0.1, 1, 10 μM) (Figure 1) after 24 hours. There were no differences in cell viability at all doses after 24-hour exposure (Figure 1). 17 β estradiol 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 compared to control (Figure 1). Cell viability in rat F98 glioblastoma cells at 48 and 72 hours decreased in all doses of 17 β estradiol compared 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 | 48 h | 72 h |
|--------|------|------|------|
|        | % cell viability (± SD) | % cell viability (± SD) | % cell viability (± SD) |
| Control | 99.83 ± 5.46a | 100.00 ± 13.83a | 100.13 ± 11.18a |
| DES (2.5 μM) | 50.67 ± 7.64b | 79.86 ± 13.11b | 67.00 ± 4.05c |
| DES (5 μM) | 40.45 ± 6.37bc | 78.00 ± 13.25b | 64.40 ± 5.64c |
| DES (10 μM) | 37.23 ± 4.67bc | 73.14 ± 5.43b | 66.40 ± 11.01c |
| DES (20 μM) | 46.23 ± 6.88bc | 99.25 ± 7.54a | 84.40 ± 4.56b |
| DES (50 μM) | 42.05 ± 7.18c | 100.88 ± 12.91a | 77.88 ± 6.62bc |
| DES (100 μM) | 46.27 ± 13.06c | 54.33 ± 28.36c | 13.17 ± 5.74a |
| P value | <0.05 | <0.05 | <0.05 |

a,b Different superscripts within the same column demonstrate significant differences (p <0.05).
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<sup>a</sup> | 6 | 79.00 ± 3.91<sup>a</sup> | 6 | 100.33 ± 14.14<sup>a</sup> | 6 |
| Progesterone (10 μM) | 76.57 ± 12.38<sup>a</sup> | 6 | 64.83 ± 21.74<sup>ab</sup> | 6 | 60.17 ± 25.64<sup>bc</sup> | 6 |
| Progesterone (20 μM) | 70.47 ± 36.10<sup>a</sup> | 6 | 50.50 ± 31.13<sup>ab</sup> | 6 | 54.60 ± 26.65<sup>bc</sup> | 6 |
| Progesterone (50 μM) | 64.00 ± 2.86<sup>a</sup> | 6 | 70.17 ± 3.49<sup>ab</sup> | 6 | 69.83 ± 3.60<sup>abc</sup> | 6 |
| Progesterone (100 μM) | 20.85 ± 11.02<sup>b</sup> | 6 | 36.80 ± 18.03<sup>b</sup> | 5 | 35.33 ± 16.53<sup>c</sup> | 6 |
| **P value**      | <0.05 | <0.05 | <0.05 |

<sup>ab</sup> 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<sup>a</sup> | 6 | 100.00 ± 13.83<sup>a</sup> | 7 | 100.17 ± 5.35<sup>a</sup> | 6 |
| 17β estradiol (0.01 μM) | 60.38 ± 7.41<sup>b</sup> | 8 | 89.50 ± 26.56<sup>ab</sup> | 4 | 68.71 ± 12.98<sup>b</sup> | 7 |
| 17βestradiol (0.1 μM) | 71.00 ± 24.51<sup>b</sup> | 8 | 74.17 ± 10.91<sup>b</sup> | 6 | 71.67 ± 6.35<sup>b</sup> | 6 |
| 17β estradiol (1 μM) | 61.00 ± 17.47<sup>b</sup> | 7 | 72.71 ± 6.53<sup>b</sup> | 7 | 70.33 ± 15.25<sup>b</sup> | 6 |
| 17β estradiol (10 μM) | 93.38 ± 17.00<sup>a</sup> | 8 | 78.25 ± 5.85<sup>b</sup> | 4 | 75.00 ± 6.44<sup>b</sup> | 5 |
| **P value**      | <0.05 | <0.05 | <0.05 |

<sup>ab</sup> 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.
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 Altık 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 (Altık 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 (Altık 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-estrol) or compounds that retain estrogenic activity (like DES-epoxide or quinone metabolites) are produced following to oral absorption 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.

**REFERENCES**

Ali Shah SI (2015). Emerging potential of parenteral estrogen as androgen deprivation therapy for prostate cancer. South Asian J Cancer, 4(2), 95–97.

Altık N, Ersöz M, Koyturk M (2011). Estradiol induces JNK-dependent apoptosis in glioblastoma cells. Oncol Lett, 2(6), 1261–1285.

Atif F, Patel NR, Yusuf S, Stein DG (2015). The Synergistic Effect of Combination Progesterone and Temozolomide on Human Glioblastoma Cells. PLoS ONE, 10(6): e0131441.

Cabrera-Muñoz E, Hernández-Hernández OT, Camacho-Arroyo J (2011). Role of progesterone in human astrocytoma growth. Curr Top Med Chem, 11(13), 1663–7.

Ding H, Wu X, Roncari L et al. (2000). Expression and regulation of neurophin-1 in human astrocytomas. Int J Cancer, 88, 584–592.

Dueñas Jiménez JM, Candalendo Arellano A, Santerre A et al. (2014). Aromatase and estrogen receptor alpha mRNA expression as prognostic biomarkers in patients with astrocytomas. J Neurooncol, 119 (2), 275–84.

Ho VK, Reinejeveld JC, Enting RH et al. (2014). Changing incidence and improved survival of gliomas. Eur J Cancer, 50(13), 2309–18.

IARC (2012). International Agency for Research on Cancer. Pharmaceuticals. Emerging potential of parenteral estrogen as androgen deprivation therapy for prostate cancer. South Asian J Cancer, 4(2), 95–97.

Kabat GC, Etgen M, Meisenzahl EM, Schijns V, Pretto C, Strik AM et al. (2014). Early steps in steroidogenesis: Intracellular cholesterol trafficking. J Lipid Res, 52, 2111–2135.

Patek S, Dibiase S, Meisenberg B et al. (2012). Phase I clinical trial assessing temozolomide and tamoxifen with concomitant radiotherapy for treatment of high-grade glioma. Int J Radiat Oncol Biol Phys, 82(2), 739–42.

Rossetti MF, Cambiasso MJ, Holubchak MA, Cabrera R (2016). Oestrogens and Progestagens: Synthesis and Action in the Brain. J Neuroendocrinol, 28 (7), 1–11.

Tang P, Roldan G, Brasher PM et al. (2006). A phase II study of carboplatin and chronic high-dose tamoxifen in patients with recurrent malignant glioma. J Neurooncol, 78, 311–6.

Tavares CB, Gomes-Braga FC, Costa-Silva DR et al. (2016). Expression of estrogen and progesterone cs in astrocytomas: a literature review. Clinics, 71 (6), 481–486.

Schins V, Prettó G, Strik AM et al. (2018). Therapeutic Immunization against Glioblastoma. Int J. Mol. Sci, 19, 2540.