Orijinal Araştırma / Original Investigation
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Effects of Trazodone on Viability in Healthy and Malignant Ovarian Cells

Trazodonun Sağlıklı ve Malignant Ovaryum Kanseri Hücrelerinde Canlılık Üzerindeki Etkileri

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ÖZET

AMAÇ: Trazodon, dizaynı 1980'li yılllara dayanan triazol piridin türevi bir antidepresandır. Başlıca, major depresyon tedavisi olmak üzere, anksiyete, uykusuzluk, şizofreni gibi psikolojik bozukluklarda kullanılmaktadır. Antidepresan kullanımının ne derece yaygın olduğu düşünüldüğünde bu grup ilaçların, hedefledikleri hastalık grubu dışında farklı patolojilerdeki etkilerinin değerlendirilmesi de anlam kazanmaktadır. Kanser, kardiyovasküler hastalıklardan sonra dünyada en sık olan ölüme yol açan hastalık grubudur. Kanser hastalarında trazodon kullanımı ve kullanımının kanser gelişimi ile ilişkisi çeşitli araştırmalarda incelenmiştir. Ancak bu çalışmalar daha çok gözlemsel ve popülasyon temelli çalışmalardır ve trazodonun kanser modelindeki moleküler etkileri net olarak bilinmemektedir.

GEREÇ VE YÖNTEM: Bu çalışmada trazodon belli bir doz aralığında (0.1-20 µM) sağlıklı over hücreleri ve over kanser hücre hatlarına uygulanmıştır. Trazodon uygulanan ve uygulanmayan hücrelerde canlılık MTT testi ile analiz edilmiştir.

BULGULAR: Elde edilen sonuçlar trazodon (0.1-20 µM) tedavisinin sağlıklı ve kanseröz over hücre canlılığı üzerinde anlamlı etki göstermediğini ortaya koymuştur.

SONUÇ: Trazadon (0.1-20 µM), hem sağlıklı hem de kanser hücrelerinin yaşayabilirliğini önemli ölçüde etkilememiştir.

Anahtar Kelimeler: trazodon, OVCAR-3, A2780, over, kanser, canlılık

ABSTRACT

OBJECTIVE: Trazodone is a triazole pyridine derivative antidepressant, the design of which dates back to the 1980s. It is mainly used in the treatment of major depression, as well as in psychological disorders such as anxiety, insomnia, and schizophrenia. Considering how widespread the use of antidepressants is, it sensible to evaluate the effects of this group of drugs in different pathologies other than the disease group they target. Cancer is the most common cause of death in the world after cardiovascular diseases. The use of trazodone in cancer patients and the relationship between the use of trazodone and the development of cancer have been reported in various studies. However, these studies are mostly observational and population-based and the molecular effects of trazodone in the cancer model are not clearly known.

MATERIALS AND METHODS: Trazodone was applied to healthy ovarian cells and OVCAR-3 and A2780 ovarian cancer cell lines at a certain dose range (0.1-20 µM). Viability in cells treated and untreated with trazodone was analyzed by MTT assay.

RESULTS: The results revealed that trazodone treatment (0.1-20 µM) did not have a significant effect on healthy and cancerous ovarian cell viability.

CONCLUSION: Trazadone (0.1-20 µM) does not significantly affect the viability of both healthy and cancer cells.

Keywords: trazodone, OVCAR-3, A2780, ovarian, cancer, viability

INTRODUCTION

Ovarian cancer is a fatal disease with poor prognosis, which is more common in women over 65 years of age (1,2). In a study evaluating the relative survival findings between 2000 and 2007, 38% survival was reported for ovarian cancer and it was stated that survival decreased with increasing age (3). In studies investigating the epidemiology of ovarian cancer, familial occurrence of this disease has been shown as an important risk factor (4). Various gene mutations, including the BRCA1 and BRCA2 genes, have also been associated with ovarian cancer (5,6). Today, surgical options and chemotherapy are used in the treatment of ovarian cancer. Although carboplatin and paclitaxel combined therapy is the primary chemotherapy approach in ovarian cancer, PARP inhibitors such as olaparib are also used in the period following primary chemotherapy, especially in patients with

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germline BRCA1 and BRCA2 or DNA repair genes mutations (7,8). Since cancer is a life-threatening fatal disease, depressive state increases in cancer patients and the use of antidepressants is on the agenda (9). From another point of view, considering the influence of antidepressant drugs on multiple signaling events, reports on the effects of antidepressant drugs against cancer have gained momentum in recent years (10). Trazodone is the first antidepressant with a triazole pyridine derivative structure designed to have different structural features from other antidepressants (11). It is used in a wide range of disorders such as anxiety, schizophrenia, insomnia and mainly major depressive disorders (12,13). In addition, antipruritic and analgesic effects have also been reported for this drug in some studies (14). Trazodone has also been suggested in the treatment of Bulimia due to its lesser side effects associated with anticholinergic signals (15). When the mechanism of action of trazodone is searched in the literature; studies elucidating the mechanisms mediating its hypnotic effects have shown that trazodone and its active metabolite, m-chlorophenylpiperazine, exhibit a partial agonistic effect at 5HT1A receptors (16) and weak antagonist activity at 5HT2 receptors (17). It has also been reported that trazodone suppresses the reuptake of 5HT to the pre-synaptic neuron, but has a low effect on norepinephrine and dopamine, which are among other catecholamines (11). There are various but limited studies in the literature on the effects of trazodone in cancer patients most of which are observational clinical studies and do not investigate the effects of trazodone on cancer cell viability and related mechanisms. In some of these studies, beneficial effects against delirium, bulimia, hot flashes, anxiety and depression in various cancers have been reported for this drug (18,19). On the other hand, some other studies reported negative effects for trazodone in cancer such as toxicity in liver, association with hepatocellular carcinoma, higher breast cancer recurrence and slightly increased cervical cancer risk (20-23). Based on these literature findings and because it is an antidepressant used in cancer patients due to several beneficial effects, we investigated the preliminary effects of trazodone on healthy and cancerous ovarian cells.

MATERIAL & METHODS

Cell Culture

OVCAR-3 (Human Ovarian cancer), IHOEC (Immortalized Human Ovarian Epithelial Cells- SV40), A2780 (Human Ovarian cancer) cell lines were grown in RPMI 1640 medium supplemented with %1 L-Glutamine, 1% penicilin/streptomycin, 10% heat inactived fetal bovine serum at 37°C, 5% CO2 incubator. IHOEC SV40 cell line was taken from Prof. Dr. Fulya Tekşen (Ankara University, Faculty of Medicine, Department of Medical Biology).

Cell Viability

To determine the effect of trazadone on the cell viability of OVCAR-3, A2780 and IHOEC cell lines, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) test was performed with some modifications of our previous study (24). For this purpoese, A2780, OVCAR-3 and IHOEC cell lines were seeded to 96 well plates (5000 cell/well). Cells were stained with trypan blue and counted on a neubauer counting chamber in order to seed an equal number of cells in the wells.

The next day, different concentrations of trazodone (0.1-20 µM) were applied to cells. Trazadone was dissolved in DMSO to prepare stock solutions. Further dilutions were made with cell culture medium. Cells not treated with trazodone were used as control group and were considered as 100% viable. The equal amount of DMSO was applied to the control group as the drug-treated cells. The DMSO concentration applied to the cells did not exceed 0.5%.

After 72 hour incubation, 20µl MTT solution was added to the wells. MTT was dissolved in PBS and 5mg/ml MTT solution was prepared and cells were incubated with MTT solution for 4 hours at 37 °C and 5%CO2. Then, the MTT-cell culture medium mixture was discarded and the formazan crystals were dissolved by adding 100 µl of DMSO. Absorbance at 550 nm was measured by microplate reader (Spectramax, Molecular Devices).

Statistical Analysis

One-way Anova variance analysis test and Tukey post hoc test was used to compare groups using GraphPad prism 9 program. Shapiro Wilk normality test was performed to determine whether the data were normally distributed. Data were expressed as the mean± standard deviation. $p<0.05$ was considered statistically significant.

RESULTS

Effects of Trazadone on the cell viability of IHOEC cells

IHOEC cells were incubated with trazadone (0.1-20μM) and trazadone did not significantly affect cell viability between 0.1-20 μM concentration range (Figure 1).

Effects of Trazadone on the cell viability of A2780 cells

A2780 cells were incubated with trazadone (0.1-20μM) and trazadone did not significantly affect cell viability between 0.1-20 μM concentration range (Figure 2).

Effects of Trazadone on the cell viability of OVCAR-3 cells

OVCAR-3 cells were incubated with trazadone (0.1-200μM) and trazadone did not significantly affect cell viability between 0.1-20 μM concentration range (Figure 3).

Figure 1. *Effect of Trazadone on the cell viability of IHOEC cells. 0.1-200μM Trazadone did not significantly affect IHOEC cell viability. (C: Control; 0.1; 0.5; 1; 5; 10; 20 depict groups treated with 0.1; 0.5; 1; 5; 10; 20 μM concentrations of trazodone).*

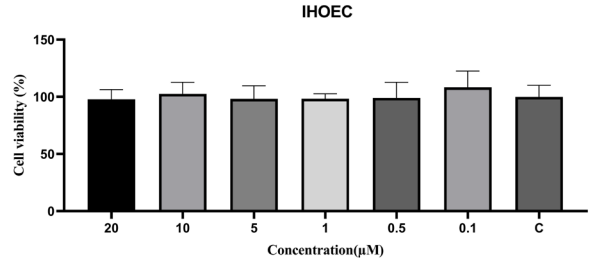


Figure 2. *Effect of Trazadone on the cell viability of A2780 cells. 0.1-20 μM Trazadone did not significantly affect A2780 cell viability. (C: Control; 0.1; 0.5; 1; 5; 10; 20 depict groups treated with 0.1; 0.5; 1; 5; 10; 20 μM concentrations of trazodone).*

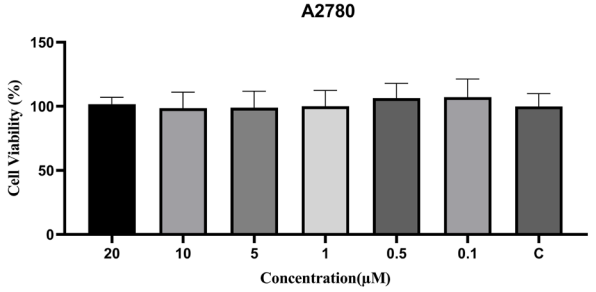
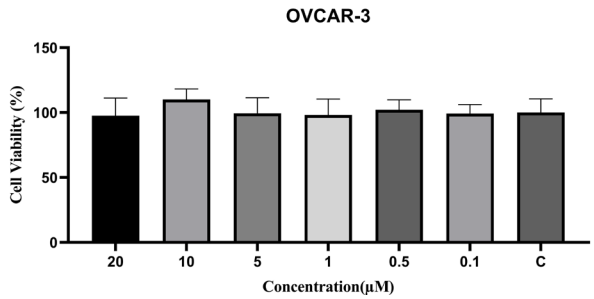


Figure 3. *Effect of Trazadone on the cell viability of OVCAR-3 cells. 0.1-200μM Trazadone did not significantly affect OVCAR3 cell viability. (C: Control; 0.1; 0.5; 1; 5; 10; 20 depict groups treated with 0.1; 0.5; 1; 5; 10; 20 μM concentrations of trazodone).*



DISCUSSION

In this study, the effects of trazodone on healthy IHOEC and cancerous OVCAR-3 and A2780 ovarian cells were investigated. With the findings, it was determined that trazodone did not have a significant effect on healthy and cancerous ovarian cells in the concentration range of 0.1-20 μM. There are very few studies in the literature investigating the effects of trazodone on cell lines. In one of these studies, trazodone was tested for its effect on P-gp (P-glycoprotein) activity and expression in Caco-2 colon cancer cell line. The dose of trazodone used in this study was reported as 25 μM, however its effect on cell viability has not been reported. In another study trazodone was tested on mouse B16 melanoma cells to test its effects on pigmentation and reported to exhibit toxicity at 140 μM concentration (25) which is higher than the highest dose we applied. On the other hand, in a study using human astrocytes, trazodone was tested at a dose range of 1nM-10 μM, and it was reported that it did not reduce unstimulated astrocyte proliferation and reversed the decrease in proliferation in LPS-TNF-α stimulated astrocytes (26). In parallel with this literature finding, we also did not find a cytotoxic effect for trazodone up to 20 μM concentration. It should be noted that the cell lines used in our study are different than astrocytes which are glial cells responsible for responses against various stimulus in the nervous system. In another study, the effects of trazodone together with doxorubicin through the eIF2alpha-P signaling pathway on A549 lung cancer cell line were investigated. Trazodone was used in this study because of its effect as a translational repressor in the eIF2 signaling pathway. The aforementioned signaling pathway interacts with another important signaling component, mTOR, and exhibits processes such as stimulation of cell migration and cell survival. Therefore, it was aimed to test the possible anti-cancer activity of trazodone in A549 cancer cells. The findings obtained in the study revealed that trazodone can exhibit anti-cancer activity with the potential to prevent migration and metastasis by suppressing the interaction between eIF2 and mTOR. Also they reported that there was no significant change between the viability of A549 cells treated with doxorubicin and doxorubucin/trazodone combination (27). This finding may suggest that trazodone preferentially effects cancer cell migration than cell viability. We also did not report anti-proliferative activity for trazodone. In addition, the concentration of trazodone used

in this study was reported to be 50 µM which is higher than our highest applied concentration. We did not test the effect of trazodone on cell migration which can be tested in further studies.

CONCLUSION

In conclusion, the findings obtained in our study revealed that trazodone did not show any toxic effect on healthy IHOEC ovarian cells at doses up to 20 µM. In addition, no anti-cancer or cancer-promoting effects were observed in A2780 and OVCAR-3 ovarian cancer cells at doses up to 20 µM of trazodone. Effects of trazodone on molecular mechanisms and specific signaling pathways may be revealed in the future.

Etik: Hücre hatları üzerinde yapılan bir çalışma olduğu için etik kurul onayı gerekmemektedir.

Since it is a study on cell lines, ethics committee approval is not required..

Yazar katkı durumu; Çalışmanın konsepti; AK, tasarım; AK, Süpervizyon; AK, Veri Toplama ve/veya İşleme; AK, AZK, HMC, HÇ, Analiz ve/veya Yorum; AK, AZK, HMC, HÇ, Literatür Tarama; AK, AZK, HMC, HÇ, El Yazması Yazma; AK, AZK.

Author contribution status; The concept of the study; AK, design; AK Supervision; AK, Data Collection and/or Processing, AK, AZK, HMC, HÇ, Analysis and/or Interpretation; AK, AZK, HMC, HÇ, Literature Search; AK, AZK, Writing Manuscript; AK, AZK.

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REFERENCES

1. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JWW, Comber H, Forman D, Bray F Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer. 2013; 49 (6):1374-1403. doi: 10.1016/j.ejca.2012.12.027

2. Oberaigner W, Minicozzi P, Bielska-Lasota M, Allemani C, De Angelis R, Mangone L, Sant M, Group EW Survival for ovarian cancer in Europe: the across-country variation did not shrink in the past decade. Acta oncologica. 2012; 51 (4):441-453

3. Sant M, Chirlaque Lopez MD, Agresti R, Sánchez Pérez MJ, Holleczek B, Bielska-Lasota M, etal. Survival of women with cancers of breast and genital organs in Europe 1999–2007: Results of the EUROCARE-5 study. European Journal of Cancer. 2015; 51 (15):2191-2205. doi: 10.1016/j.ejca.2015.07.022

4. Nguyen HN, Averette HE, Janicek M Ovarian carcinoma: a review of the significance of familial risk factors and the role of prophylactic oophorectomy in cancer prevention. Cancer. 1994; 74 (2):545-555

5. Lynch HT, Casey MJ, Snyder CL, Bewtra C, Lynch JF, Butts M, Godwin AK Hereditary ovarian carcinoma: heterogeneity, molecular genetics, pathology, and management. Molecular oncology. 2009; 3 (2):97-137

6. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE Risks of cancer in BRCA1-mutation carriers. The Lancet. 1994; 343 (8899):692-695

7. Norquist BM, Brady MF, Harrell MI, Walsh T, Lee MK, Gulsuner S, Bernards SS, Casadei S, Burger RA, Tewari KS Mutations in homologous recombination genes and outcomes in ovarian carcinoma patients in GOG 218: an NRG oncology/gynecologic oncology group study. Clinical Cancer Research. 2018; 24 (4):777-783

8. Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, Mannel RS, DeGeest K, Hartenbach EM, Baergen R Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a Gynecologic Oncology Group study. Journal of clinical oncology. 2003; 21 (17):3194-3200

9. Ostuzzi G, Matcham F, Dauchy S, Barbui C, Hotopf M Antidepressants for the treatment of depression in people with cancer. Cochrane Database Syst Rev. 2018; 4 (4):Cd011006. doi:10.1002/14651858.CD011006.pub3

10. Zheng Y, Chang X, Huang Y, He D The application of antidepressant drugs in cancer treatment. Biomedicine & Pharmacotherapy. 2023; 157:113985. doi: 10.1016/j.biopha.2022.113985

11. Jackson EA, Cardoni AA, Rawls WN Trazodone (Desyrel, Mead-Johnson Pharmaceutical Division). Drug Intelligence & Clinical Pharmacy. 1982; 16 (1):7-13. doi:10.1177/106002808201600102

12. Haria M, Fitton A, McTavish D Trazodone. Drugs & Aging. 1994; 4 (4):331-355. doi:10.2165/00002512-199404040-00006

13. Earl DC, Van Tyle KM New pharmacologic agents for insomnia and hypersomnia. Curr Opin Pulm Med. 2020; 26 (6):629-633. doi:10.1097/mcp.0000000000000722

14. Belinskaia DA, Belinskaia MA, Barygin OI, Vanchakova NP, Shestakova NN Psychotropic Drugs for the Management of Chronic Pain and Itch. Pharmaceuticals (Basel). 2019; 12 (2). doi:10.3390/ph12020099

15. Pope HG, Jr., Hudson JI Antidepressant medication in the treatment of bulimia nervosa. Psychopathology. 1987; 20 Suppl 1:123-129. doi:10.1159/000284531

16. Odagaki Y, Toyoshima R, Yamauchi T Trazodone and its active metabolite m-chlorophenylpiperazine as partial agonists at 5-HT1A receptors assessed by [35S] GTPγS binding. Journal of Psychopharmacology. 2005; 19 (3):235-241

17. Marek GJ, McDougle CJ, Price LH, Seiden LS A comparison of trazodone and fluoxetine: implications for a serotonergic mechanism of antidepressant action. Psychopharmacology (Berl). 1992; 109 (1-2):2-11. doi:10.1007/bf02245475

18. Maeda I, Inoue S, Uemura K, Tanimukai H, Hatano Y,

Yokomichi N, Amano K, Tagami K, Yoshiuchi K, Ogawa A, Iwase S Low-Dose Trazodone for Delirium in Patients with Cancer Who Received Specialist Palliative Care: A Multicenter Prospective Study. J Palliat Med. 2021; 24 (6):914-918. doi:10.1089/jpm.2020.0610

19. Tanimukai H, Murai T, Okazaki N, Matsuda Y, Okamoto Y, Kabeshita Y, Ohno Y, Tsuneto S An observational study of insomnia and nightmare treated with trazodone in patients with advanced cancer. Am J Hosp Palliat Care. 2013; 30 (4):359-362. doi:10.1177/1049909112452334

20. Voican CS, Corruble E, Naveau S, Perlemuter G Antidepressant-induced liver injury: a review for clinicians. Am J Psychiatry. 2014; 171 (4):404-415. doi:10.1176/appi.ajp.2013.13050709

21. Chen VC-H, Lin C-F, Hsieh Y-H, Liang H-Y, Huang K-Y, Chiu W-C, Lee Y, McIntyre RS, Chan H-L Hepatocellular carcinoma and antidepressants: a nationwide population-based study. Oncotarget. 2016; 8 (18)

22. Chubak J, Bowles EJ, Yu O, Buist DS, Fujii M, Boudreau DM Breast cancer recurrence in relation to antidepressant use. Cancer Causes Control. 2016; 27 (1):125-136. doi:10.1007/s10552-015-0689-y

23. Chan H-L, Hsieh Y-H, Lin C-F, Liang H-Y, Huang K-Y, Chiu W-C, Lee Y, McIntyre RS, Chen VC-H Invasive Cervical Cancer and Antidepressants: A Nationwide Population-Based Study. Medicine. 2015; 94 (42)

24. Ozkan T, Hekmatshoar Y, Karabay AZ, Koc A, Altinok Gunes B, Karadag Gurel A, Sunguroglu A Assessment of azithromycin as an anticancer agent for treatment of imatinib sensitive and resistant CML cells. Leuk Res. 2021; 102:106523. doi:10.1016/j.leukres.2021.106523

25. Chang TS, Lin VC Melanogenesis inhibitory activity of two generic drugs: cinnarizine and trazodone in mouse B16 melanoma cells. Int J Mol Sci. 2011; 12 (12):8787-8796. doi:10.3390/ijms12128787

26. Daniele S, Zappelli E, Martini C Trazodone regulates neurotrophic/growth factors, mitogen-activated protein kinases and lactate release in human primary astrocytes. J Neuroinflammation. 2015; 12:225. doi:10.1186/s12974-015-0446-x

27. Harvey RF, Pöyry TAA, Stoneley M, Willis AE Signaling from mTOR to eIF2α mediates cell migration in response to the chemotherapeutic doxorubicin. Sci Signal. 2019; 12 (612). doi:10.1126/scisignal.aaw6763