Journal of Biological Studies
ISSN 2209-2560

Research Full-Text Paper

The tricyclic antidepressant amitriptyline is cytotoxic to human breast cancer (MCF7) cells

Atefe Dehghani[1]*, Afrim Tabaku[2], Sahar Eshghjoo[3]

[1] *Member of Young Researchers and Elite Club, Department of Biology, Faculty of Basic Sciences, Hamedan Branch, Islamic Azad University, Hamedan, Iran*
[2] *Pharmacotherapeutics Research Centre, Faculty of Medical Sciences, University Aldent, Tirana-Albania*
[3] *Department of Microbial Pathogenesis and Immunology, College of Medicine, Texas A&M Health Science Center, Texas, USA*

Received January 13,2022; Accepted February 7,2022; Published online April 10,2022

Abstract

Although many studies suggest that antidepressants can affect cancer cell growth and proliferation, the results are still challenging. The aim of this study was to evaluate the cytotoxic effect of amitriptyline on MCF7 viability *in vitro*. Breast cancer (MCF7) cells were divided into control group and groups treated with 1250, 625, 312.5, 156.25, 78.125, 39.0625 µg/ml of amitriptyline. 24 and 48 hours after treatment, cell viability was assessed by MTT method. Data were analyzed using one-way analysis of variance. Cell viability significantly decreased in MCF7 cells treated with amitriptyline (39.06, 78.12, 156.25, 312.50, 625 and 1250 µg/ml) compared to control group 24 and 48 hours after treatment; however, treatment of MCF7 cells with 39.06 µg/ml of amitriptyline did not significantly change the cell viability compared with control group. Calculated IC50 for 48 hours was more than IC50 calculated for amitriptyline during 24 hours of treatment. Our findings indicated that amitriptyline has significant cytotoxic effects on breast cancer (MCF7) cells *in vitro*. Increased treatment duration leads to increased cytotoxic capability of amitriptyline on MCF7 cells.

Keywords: Amitriptyline, MCF7, Viability

*e-mail: atefedehqani1999@gmail.com

1 Introduction

In 2015, about 90.5 million people were diagnosed with cancer (Lipton et al., 2016). From 2019, about 18 million new cases occur annually (Shu et al., 2020). It causes about 8.8 million deaths (15.7% of deaths) annually (Wang et al., 2016). A significant number of cancer patients become clinically depressed (Chochinov, 2001). Non-pathological grief may be a natural response to a cancer diagnosis, however, stress beyond patients' coping mechanisms may lead to major depressive disorder. There will be urgent need to identify and treat depression in cancer patients in order to increase quality of life and reduce mortality. The interventions used vary from patient to patient, but may include psychosocial therapy or medication (Smith, 2015). In the context of cancer, such as breast cancer, depression is associated with increased complications, longer hospital stays, and more general disability. Therefore, prompt diagnosis and effective treatment are essential. Several studies have demonstrated the efficacy of tricyclic antidepressants (TCAs) such as amitriptyline in these conditions (Pezzella et al., 2001). Amitriptyline is the most common analgesic drug used in cancer patients with neuropathic pain (Mercadante et al., 2002). In addition to its antidepressant effect, amitriptyline has been reported to cause the highest cell damage in certain cancer cells. As a result, amitriptyline is emerging as a new drug for testing anticancer therapy (Cordero et al., 2010). Amitriptyline may also play a supportive role in the efficacy of anticancer drugs (Kulaksiz-Erkmen et al., 2013). Most of the previous studies have examined the effect of amitriptyline on cancer exacerbation in cancer patients or the relief of neuropathic pain after cancer treatment with amitriptyline and few studies have focused on anticancer effects of amitriptyline *in vitro*. Accordingly, due to the fact that most of the patients with breast cancer use antidepressants such as amitriptyline to treat depression (Fisch, 2004) and the use of amitriptyline may have anticancer effects on cancer cells (Möslinger-Gehmayr et al., 2000), we investigated the tricyclic antidepressant amitriptyline cytotoxic effects on human breast cancer (MCF7) cells *in vitro*.

2 Materials and Methods

2. 1. Drugs and cells

MCF7 cancer cells were purchased from Pasteur Institute of Iran. Amitriptyline was purchased from Abureyhan pharmaceutical company (Iran, Tehran) and stored at room temperature away from moisture, heat and light.

2. 2. Cell culture

Eagle's MEM (EMEM), supplemented with 10% FBS and 1% penicillin/streptomycin, non-essential amino acids (0.1 mM), insulin (10 ug/mL) and sodium pyruvate (1 mM) was used for culturing MCF7 cells. The culture medium was maintained at incubator with temperature at 37°C in humidified, concentrated CO2 (5%) atmosphere. As MCF-7 cells reached approximately 85-90% confluency, medium was remove and the cells were rinsed twice with PBS. 2-3 mL of warm (37°C) 0.25% Trypsin was added to detach the cell layer. Cells were observed under an

inverted microscope to examine the cell dispersion. After cell layer detachment, Trypsin was neutralized by adding 10mL of complete growth medium to the flask. Cells were suspended by gently pipetting and were centrifuged for 5 minutes at 125 x g to pellet cells. Trypsin/growth medium suspension was aspirated from tube. MCF-7 cell pellet were resuspended in 10 mL fresh growth medium. MCF-7 cells were plated into a new flask containing complete growth medium and incubated at 37°C in humidified 5% CO2 atmosphere.

2. 3. Treatment

Cells were divided into 7 groups: control (untreated) group, and groups treated with 1250, 625, 312.5, 156.25, 78.125, and 39.0625 μg / ml amitriptyline for 24h and 48h.

2. 4. MTT assay

In order to evaluate the cytotoxic effects of amitriptyline on MCF7 cells, MTT assay was used. In this regard, media was discarded from cell cultures. For adherent cells, carefully aspirate the media. For suspension cells, spin the 96 well plate at 1,000 xg, 4°C for 5 minutes in a microplate-compatible centrifuge and carefully aspirate the media. 50 μL of serum-free media and 50 μL of MTT solution were added into each well. The plate was incubated at 37°C for 3 hours. After incubation, 150 μL of MTT solvent was added into each well. Plate was wraped in foil and shaked on an orbital shaker for 15 minutes. Occasionally, the liquid was pipetted to fully dissolve the MTT formazan. Absorbance was read at OD=590 nm. Percentage cytotoxicity was calculated with the following equation:

$$\% \text{ cytoxicity} = (100 \text{ x } (\text{control - sample}))$$

IC50 of amitriptyline calculated from curve constructed by plotting MCF7 cell viability (%) versus amitriptyline 24 and 48 hours after treatment (Li et al., 2012).

2. 5. Statistical analysis

Data were analyzed using SPSS21 software (IBM, USA) and one-way analysis of variance followed by Tukey's post hoc test. Differences between groups were considered significant at the level of $p<0.05$.

3 Results and Discussions

Cell viability significantly decreased in MCF7 cells treated with amitriptyline (39.06, 78.12, 156.25, 312.50, 625 and 1250 μg/ml) compared to control group 24 and 48 hours after treatment (Figure 1 and 2); however, treatment of MCF7 cells with 39.06 μg/ml of amitriptyline did not significantly change the cell viability compared with control group.

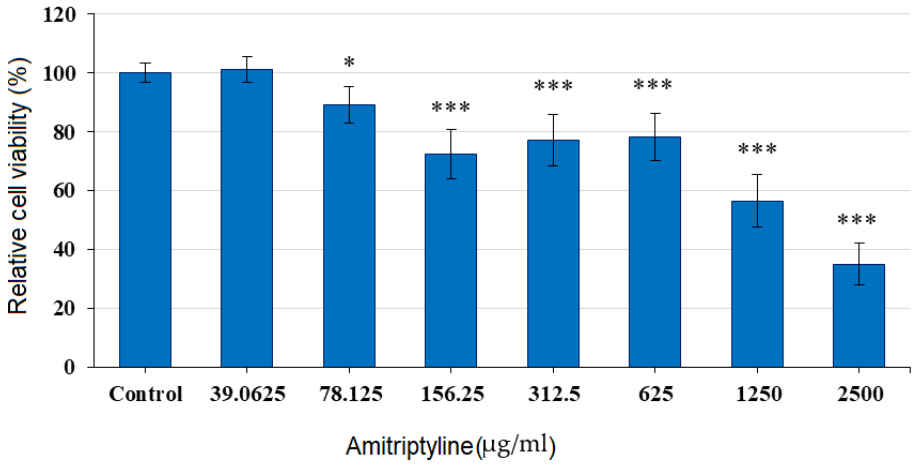

Figure 1. Viability of MCF7 cells treated with different concentrations of amitriptyline 24 hours after treatment. * Shows a significant difference compared to the control group. (*:p<0.05), (***:p<0.001)

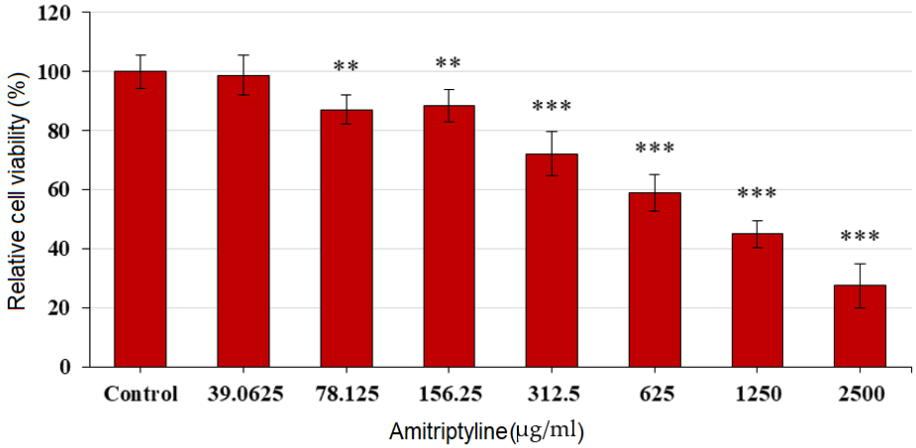

Figure 2. Viability of MCF7 cells treated with different concentrations of amitriptyline 48 hours after treatment. * Shows a significant difference compared to the control group. (**:p<0.01), (***:p<0.001)

The half-maximal inhibitory concentration (IC50) of amitriptyline was calculated by linear approximation regression of the percentage survival versus the amitriptyline concentration. The results showed that the IC50 value was 1321 µg/ml for amitriptyline 24 hours after treatment (Figure 3).

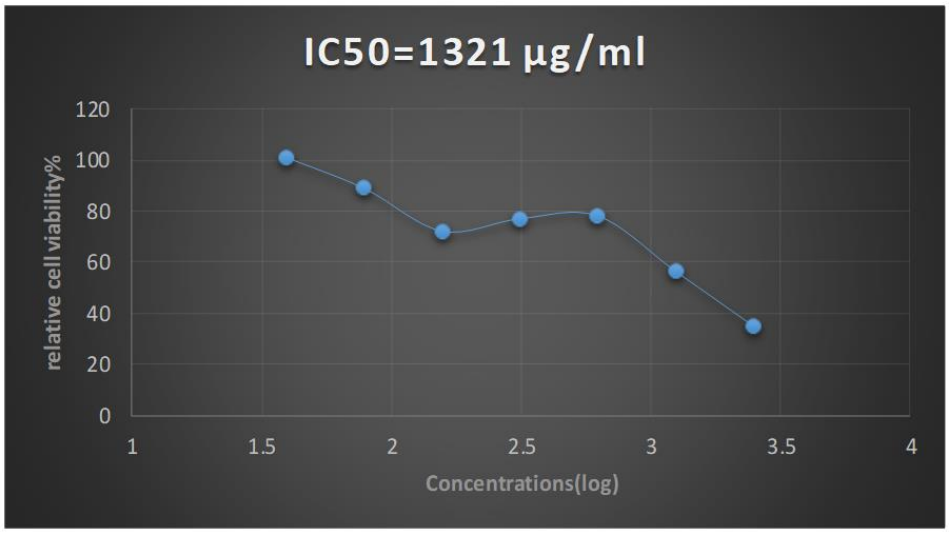

Figure 3. IC50 of amitriptyline calculated from curve constructed by plotting MCF7 cell viability (%) versus amitriptyline 24 hours after treatment.

The IC50 value 881 µg/ml for amitriptyline 48 hours after treatment showing a significant decrease in IC50 value compared to amitriptyline IC50 during 24 hours of treatment indicating that the cytotoxic effect of the drug significantly increases with increasing of treatment duration (Figure 4).

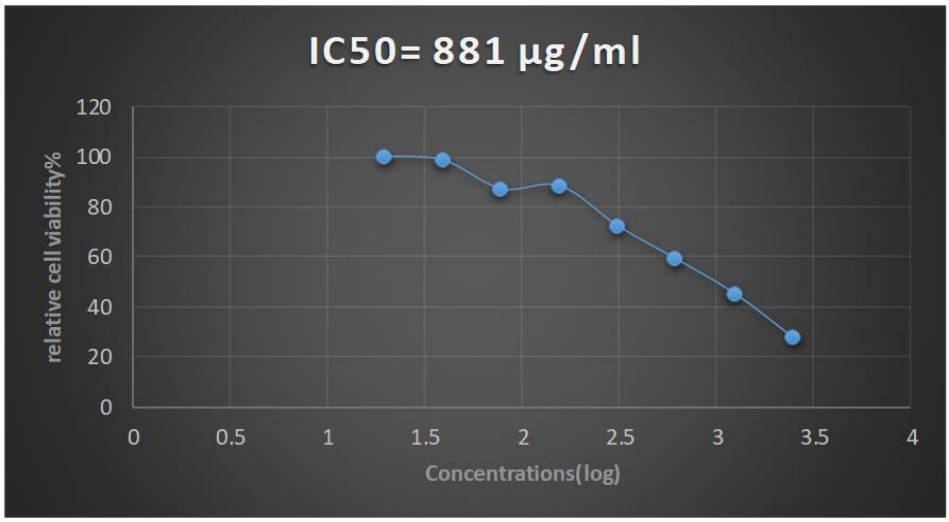

Figure 4. Calculated IC50 value of amitriptyline 48 hours after treatment.

The results of this study show that amitriptyline in low doses can significantly reduce the viability of MCF7 cancer cells. Consistent with our finding it has been shown that the antidepressant fluoxetine suppresses glioblastoma cells proliferation by inducing calcium-dependent apoptosis (Liu et al., 2015). In another study that examined the effect of different antidepressants on cell viability and proliferation of human colorectal cancer cell lines, paroxetine and sertraline have been reported to inhibit the colorectal cancer cell viability and proliferation in a dose-dependent manner (Gil-Ad et al., 2008). Anticancer effects of tricyclic antidepressant amitriptyline also have been reported in recent studies. Amitriptyline has been

shown to induce cell death on myeloma cells (Mao et al., 2011). The antidepressant paroxetine has been reported to induce apoptosis in MCF-7 cells (Cho et al., 2019). The clinical research indicates that evaluation of anti-depressants as adjunct therapeutics with chemotherapy may have a translational effect for lung cancer patient (Zingone et al., 2017). By contrast, there are findings showing that antidepressant drugs may increase cancer cells growth and proliferation. The antidepressant desipramine has been shown that promotes breast tumor progression in association with altered collagen structure (Szpunar et al., 2013).

The antiproliferative activity of amitriptyline may be mediated by mitochondrial inhibitors and by disrupting the cell cycle (Geeraerts et al., 2021). Amitriptyline is also able to inhibit tumor-specific proteins (TCTP) and therefore possibly exerts its anti-cancer effects by inhibiting the expression of TCTPs (Baú-Carneiro et al., 2022).

4 Conclusion

The results of this study revealed that amitriptyline has significant cytotoxic effects on breast cancer (MCF7) cells *in vitro* and the treatment duration plays a role to increase the cytotoxic effect of the drug on breast cancer cells.

Acknowledgments

This research was supported by Global Research, Education and Events Network (GREEN) and approved by International Association of Scientists (IAS).

Conflict of interests

The authors state that there are no conflicts of interests regarding the publication of this article.

References

Baú-Carneiro, J. L., Sumida, I. A. G., Gallon, M., Zaleski, T., Boia-Ferreira, M., & Cavassin, F. B. (2022). Sertraline repositioning: an overview of its potential use as a chemotherapeutic agent after four decades of tumor reversal studies. *Translational Oncology, 16*, 101303. https://doi.org/10.1016/j.tranon.2021.101303

Cho, Y.W., Kim, E.J., Nyiramana, M.M., Shin, E.J., Jin, H., Ryu, J.H., Kang, K.R., Lee, G.W., Kim, H.J., Han, J., & Kang, D. (2019). Paroxetine induces apoptosis of human breast cancer MCF-7 cells through Ca2+-and p38 MAP kinase-dependent ROS generation. *Cancers*, *11*(1), 64. https://doi.org/10.3390/cancers11010064

Chochinov, H. M. (2001). Depression in cancer patients. *The lancet oncology, 2*(8), 499-505.

https://doi.org/10.1016/S1470-2045(01)00456-9

Cordero, M.D., Sánchez-Alcázar, J.A., Bautista-Ferrufino, M.R., Carmona-López, M.I., Illanes, M., Ríos, M.J., Garrido-Maraver, J., Alcudia, A., Navas, P., & de Miguel, M. (2010). Acute oxidant damage promoted on cancer cells by amitriptyline in comparison with some common chemotherapeutic drugs. *Anti-cancer drugs, 21*(10), 932-944. https://doi.org/10.1097/cad.0b013e32833ed5f7

Fisch, M. (2004). Treatment of depression in cancer. *JNCI Monographs, 2004*(32), 105-111. https://doi.org/10.1093/jncimonographs/lgh011

Geeraerts, S.L., Kampen, K.R., Rinaldi, G., Gupta, P., Planque, M., Louros, N., Heylen, E., De Cremer, K., De Brucker, K., Vereecke, S., & De Keersmaecker, K. (2021). Repurposing the Antidepressant Sertraline as SHMT Inhibitor to Suppress Serine/Glycine Synthesis–Addicted Breast Tumor Growth. Molecular Cancer Therapeutics, 20(1), 50-63. https://doi.org/10.1158/1535-7163.mct-20-0480

Gil-Ad, I., Zolokov, A., Lomnitski, L., Taler, M., Bar, M., Luria, D., Ram, E., & Weizman, A. (2008). Evaluation of the potential anti-cancer activity of the antidepressant sertraline in human colon cancer cell lines and in colorectal cancer-xenografted mice. *International journal of oncology*, *33*(2), 277-286. https://doi.org/10.3892/ijo_00000007

Kulaksiz-Erkmen, G., Dalmizrak, O., Dincsoy-Tuna, G., Dogan, A., Ogus, I. H., & Ozer, N. (2013). Amitriptyline may have a supportive role in cancer treatment by inhibiting glutathione S-transferase pi (GST-π) and alpha (GST-α). *Journal of enzyme inhibition and medicinal chemistry, 28*(1), 131-136. https://doi.org/10.3109/14756366.2011.639017

Li, Y., Huang, W., Huang, S., Du, J., & Huang, C. (2012). Screening of anti-cancer agent using zebrafish: comparison with the MTT assay. *Biochemical and biophysical research communications, 422*(1), 85-90. https://doi.org/10.1016/j.bbrc.2012.04.110

Liu, K.H., Yang, S.T., Lin, Y.K., Lin, J.W., Lee, Y.H., Wang, J.Y., Hu, C.J., Lin, E.Y., Chen, S.M., Then, C.K., & Shen, S. C. (2015). Fluoxetine, an antidepressant, suppresses glioblastoma by evoking AMPAR-mediated calcium-dependent apoptosis. *Oncotarget*, *6*(7), 5088. doi: 10.18632/oncotarget.3243

Lipton, R. B., Schwedt, T. J., & Friedman, B. W. (2016). GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet. 388*(10053): 1545-1602. https://doi.org/10.1016/s0140-6736(16)31678-6.

Mao, X., Hou, T., Cao, B., Wang, W., Li, Z., Chen, S., Fei, M., Hurren, R., Gronda, M., Wu, D., & Schimmer, A. D. (2011). The tricyclic antidepressant amitriptyline inhibits D-cyclin transactivation and induces myeloma cell apoptosis by inhibiting histone deacetylases: in vitro and in silico evidence. *Molecular pharmacology*, *79*(4), 672-680. https://doi.org/10.1124/mol.110.068122

Mercadante, S., Arcuri, E., Tirelli, W., Villari, P., & Casuccio, A. (2002). Amitriptyline in

neuropathic cancer pain in patients on morphine therapy: a randomized placebo-controlled, double-blind crossover study. *Tumori Journal, 88*(3), 239-242. http://dx.doi.org/10.1177/030089160208800310

Möslinger-Gehmayr, R., Zaninelli, R., Contu, A., Oberhoff, C., Gutschow, K., Schindler, A. E., & Staab, H. J. (2000). A double-blind comparative study of the effectiveness and tolerance of paroxetine and amitriptyline in treatment of breast cancer patients with clinically assessed depression. *Zentralblatt fur Gynakologie, 122*(4), 195-202. PMID: 10795116. https://pubmed.ncbi.nlm.nih.gov/10795116/

Pezzella, G., Moslinger-Gehmayr, R., & Contu, A. (2001). Treatment of depression in patients with breast cancer: a comparison between paroxetine and amitriptyline. *Breast cancer research and treatment, 70*(1), 1-10. https://doi.org/10.1023/a:1012518831494

Shu, X. E., Swanda, R. V., & Qian, S. B. (2020). Nutrient control of mRNA translation. *Annual Review of Nutrition*, *40*, 51-75. https://doi.org/10.1146/annurev-nutr-120919-041411

Smith, H. R. (2015). Depression in cancer patients: Pathogenesis, implications and treatment. Oncology letters, 9(4), 1509-1514. https://doi.org/10.3892/ol.2015.2944

Szpunar, M. J., Burke, K. A., Dawes, R. P., Brown, E. B., & Madden, K. S. (2013). The antidepressant desipramine and α2-adrenergic receptor activation promote breast tumor progression in association with altered collagen structure. *Cancer Prevention Research*, *6*(12), 1262-1272. https://doi.org/10.1158/1940-6207.CAPR-13-0079

Wang, H., Naghavi, M., Allen, C., Barber, R.M., Bhutta, Z.A., Carter, A., Casey, D.C., Charlson, F.J., Chen, A.Z., Coates, M.M., & Bell, M. L. (2016). Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. The lancet, 388(10053), 1459-1544.4. https://doi.org/10.1016/S0140-6736(16)31012-1

Zingone, A., Brown, D., Bowman, E. D., Vidal, O. M., Sage, J., Neal, J., & Ryan, B. M. (2017). Relationship between anti-depressant use and lung cancer survival. *Cancer treatment and research communications*, *10*, 33-39. https://doi.org/10.1016/j.ctarc.2017.01.001