BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e201148

Article

Anti-proliferative effects of paroxetine alone or in combination with sorafenib in HepG2 cells

Yaprak Donmez Cakil[1,2], Zeynep Gunes Ozunal[2,3], Damla Gokceoglu Kayali[2], Ranan Gulhan Aktas[1,2], Esra Saglam[3]

[1]Department of Histology and Embryology, Faculty of Medicine, Maltepe University, Istanbul, Turkey,
[2]Cancer and Stem Cell Research Center, Faculty of Medicine, Maltepe University, Istanbul, Turkey,
[3]Department of Medical Pharmacology, Faculty of Medicine, Maltepe University, Istanbul, Turkey

Hepatocellular carcinoma (HCC) is a common cause of cancer-related death. Sorafenib is the first approved drug for the treatment of advanced HCC. Depression is frequent in cancer patients. Moreover, sorafenib might exert depression as an adverse drug reaction and paroxetine, a selective serotonin reuptake inhibitor, is a recommended pharmacotherapy. This study aimed to investigate the potential synergistic effects of paroxetine and sorafenib on HepG2 cell proliferation and death. Paroxetine and sorafenib were administered to HepG2 cells as single-agents or in combination. Cell viability was determined with XTT cell viability assay. Cellular apoptosis and DNA content were assessed by flow cytometry. The expression of anti-apoptotic Bcl-2 was examined by immunofluorescence confocal microscopy. A lower dose of sorafenib was found to be required to inhibit cell proliferation when in combination with paroxetine. Similarly, the coadministration enhanced cellular apoptosis and resulted in cell cycle arrest. Confocal imaging revealed a remarkably lower cell density and increased expression of Bcl-2 following combined treatment of paroxetine with sorafenib. To our knowledge, this is the first study demonstrating the synergistic effect of paroxetine and sorafenib in HCC and might provide a potentially promising therapeutic strategy.

Keywords: Paroxetine, Sorafenib, Pharmacotherapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the leading primary malignancy of the liver and an important cause of cancer-related death. Hepatitis B and C virus infection, and nonalcoholic steatohepatitis, are associated with a high risk for developing HCC, while the majority of cases are observed in cirrhotic patients (Ghouri, Mian, Rowe, 2017; Villanueva, 2019). No systemic agent has been found that improved the survival of patients with HCC until the emergence of the oral multi-kinase inhibitor sorafenib (Bangaru, Marrero, Singal, 2020). Sorafenib suppresses tumor cell proliferation, promotes apoptosis, and decreases angiogenesis by inhibiting receptor tyrosine kinase signaling and downstream Raf serine/threonine kinase activity (Zhu *et al*., 2017). Currently, sorafenib is within the first-line systemic therapy options in patients with advanced HCC, where curative treatments, such as transplant or surgical resection, are not a choice (Llovet *et al*., 2021). It was established as a reference drug for advanced HCC based on the improved overall survival of several months depending on the subgroups of patients in Phase III studies (Llovet *et al*., 2008; Cheng *et al*., 2009). Two recent retrospective studies have demonstrated an increased survival rate of advanced HCC patients after sorafenib treatment also in the real-life clinical setting (Longo *et al*., 2018; Huang *et al*., 2019). Unfortunately, the effectiveness of the approved drugs for HCC is inadequate. Moreover, there are safety concerns. The associated adverse effects, such as diarrhea, rash, hand-foot syndrome, and high blood pressure, may require a decrease in dosage limiting the efficacy of sorafenib treatment (Hampton, 2007; Zhu *et al*., 2017). Another

***Correspondence:** Y. Donmez Cakil. Department of Histology and Embryology. Faculty of Medicine. Marmara Eğitim Köyü 34857 Maltepe, ISTANBUL, Turkey. ORCID: https://orcid.org/0000-0002-4605-1167

concern about sorafenib therapy is drug resistance (Gauthier, Ho, 2013). Approximately 30% of patients were reported to show different sensitivity to the treatment, pointing to inherent or acquired sorafenib resistance (Cabral, Tiribelli, Sukowati, 2020). Several basic and clinical studies have suggested the combined use of sorafenib with other drugs to lower its onset concentration and achieve a higher tumor inhibition rate with fewer adverse effects (Gauthier, Ho, 2013; Zhu *et al*., 2017).

Depression may develop in more than 10% of cancer patients (Smith, 2015). Moreover, sorafenib might cause depression as a central nervous system-related adverse effect (Bhojani*et al*., 2008). Selective serotonin reuptake inhibitors (SSRIs) are frequently used in the treatment of depression. Among them, paroxetine, a well-tolerated phenylpiperidine derivative, is the most widely-prescribed serotonin transporter antagonist and is a recommended pharmacotherapy for depression (Bourin, Chue, Guillon, 2001; Davis *et al*., 2016). Aside from its roles in psychiatry, a distinct role in cancer is also suggested. There are several in vitro studies that have demonstrated its cytotoxic activities in cancer. Paroxetine was reported to induce apoptosis in osteosarcoma cells (Chou, He, Jan, 2007), OC2 human oral cancer cells (Fang *et al*., 2011), human lung cancer cells (Rosetti *et al*., 2006), human T leukemia cells (Amit *et al*., 2009), human breast cancer cells (Cho *et al*., 2019), and rat glioma and human neuroblastoma cells (Levkovitz *et al*., 2005). A population-based case-control study with 59,859 HCC cases demonstrated that SSRIs, including paroxetine, were associated with lower HCC risk (Chan *et al*., 2018).

This study aimed to set up translational research to evaluate the combined effect of paroxetine and sorafenib in the HepG2 HCC cell line. Herein, cell proliferation, apoptosis, and cell cycle distribution were investigated and it was sought to determine the potential synergistic anti-cancer effect of a paroxetine-sorafenib combination.

MATERIAL AND METHODS

Drugs

Paroxetine (Sigma-Aldrich, USA) and sorafenib (LC Laboratories, USA) powders were solubilized at a concentration of 10 mmol/L in water and DMSO, respectively. The stock solutions were freshly diluted in the cell culture medium on the day of the experiment.

Cell culture

The HepG2 HCC cell line was obtained from ATCC (American Type Culture Collection, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Biosera LM-T1720/100, France) supplemented with 10% (v/v) fetal bovine serum (PAN-Biotech GmbH, Germany) and 1% (v/v) penicillin-streptomycin (PAN-Biotech GmbH, Germany) at 37 °C in a humidified atmosphere of 5% CO_2.

Cell viability assay with XTT reagent

The XTT Cell Proliferation Assay Kit (Biological Industries, USA) was used to measure cell viability. The cells were stained with trypan blue, counted, and seeded as 10,000 cells/well in a 96-well plate dish. On the following day, paroxetine or sorafenib was added to the cells as single agents or in combination. The highest concentrations of paroxetine and sorafenib were 25 and 50 µM, respectively. The drugs were diluted in DMEM with a rate of ¾ and added to the cells. XTT reagent was added after 24 h of incubation, and the optical density of the soluble product was measured at 500 nm with a Synergy Microplate Reader (BioTek, Japan).

The half-maximal inhibitory concentration (IC50) was determined for each drug by non-linear regression analysis using MS Excel software. GraphPad Prism V.8.01 (San Diego, CA, USA) was used to plot the bar graphs.

SynergyFinder (https://synergyfinder.fimm.fi), was used to determine the paroxetine-sorafenib combination effect. The degree of a drug combination effect can be visualized (Ianevski et al., 2017). The application calculates the synergy score based on the Bliss model (Bliss, 1939).

Detection of apoptosis using flow cytometry

Following incubation with IC50 doses of the drugs for 24 h as single agents or in combination, the cells were harvested and prepared for Annexin V/propidium iodide (PI)

apoptosis detection assay according to the manufacturer's instructions (BD Pharmingen, BD Biosciences, USA). The cells were resuspended in the binding buffer at a concentration of 1×10^6 cells/mL, and then stained with Annexin V-APC and PI. After an incubation for 15 min, the cells were measured in a BD AccuriC6 Plus flow cytometer (BD Biosciences, USA). The flow cytometric analyses were carried out using FlowJo V.10.6.1 (USA). Live cells are both Annexin V and PI negative, the cells that are in early apoptosis are Annexin V positive and PI negative, and the cells that are in late apoptosis or already dead are both Annexin V and PI positive. The cells that undergo cell death in a way other than apoptosis are Annexin V negative and PI positive.

Cell cycle analysis

DNA contents of the cells after treatment with IC50 doses of the drugs for 24 h were evaluated using a BD Cycletest™ Plus DNA Reagent Kit according to the manufacturer's instructions (BD Pharmingen, BD Biosciences, USA). After trypsinization, the cells were washed, the supernatant was discarded, and the cell pellet was mixed with Solution A. Solutions B and C were added after subsequent incubations. The cell cycle distribution was analyzed in a BD AccuriC6 Plus flow cytometer (BD Biosciences, USA). The results were analyzed using FCS Express 7 Research Edition (USA).

Immunostaining and confocal microscopy

After the administration of IC50 doses of the drugs for 24 h as single agents or in combination, the cells were fixed in formalin for 15 min at room temperature (RT). They were washed with PBS and incubated in PBS containing 2% BSA for 15 min at RT to block nonspecific bindings. Subsequently, the cells were incubated overnight with mouse anti-human Bcl-2 antibody at a dilution of 1:100 (Biocare, US). The next day, the cells were washed and probed with goat anti-mouse IgG DyLight 488 at a dilution of 1:200 (Thermo Scientific, USA) for 30 min at RT. The nuclei were counterstained with 1 mg/mL of Hoechst 33258 (Thermo Scientific) solution. Images were taken with a confocal scanning microscope (Zeiss LSM 700, Germany).

Statistical analyses

All data were representative of at least three independent experiments and expressed as the mean ± standard error of the mean (SEM). Immunofluorescence quantification data were evaluated by one-way analysis of variance (ANOVA), and apoptosis and cell cycle assays were analyzed by two-way ANOVA tests using GraphPad Prism V.8.01. An α of 0.05 was used as the cut off for significance and post hoc Tukey analyses were carried out.

RESULTS AND DISCUSSION

Evaluation of paroxetine-sorafenib combination for cell viability

First, to examine the cytotoxicity of paroxetine as a single agent, HepG2 cells were administered for 24 h with increasing concentrations of paroxetine, with the highest concentration at 25 µM. Figure 1A demonstrates the anti-proliferative effects of paroxetine in a dose-dependent manner. The IC50 was calculated as 10.2 ± 1 µM. The results correlated with those reported in prior studies. Paroxetine has been reported to reduce cell proliferation with an IC50 value of 7.3 µM in HepG2 Cells (Kuwahara *et al*., 2015), and an IC50 value of 18 µM in malignant T cells (Jurkat) (Amit *et al.,* 2009). The inhibitory effect of sorafenib on cell viability was determined previously and the IC50 value was reported as 17.8 ± 1.6 µM in HepG2 cells (Ozunal *et al*., 2019).

Next, a possible synergistic effect of paroxetine and sorafenib was investigated. To analyze the type of interaction between the paroxetine and sorafenib when used in combination, 'SynergyFinder', which is a web based application was utilized. The Loewe additivity and the Bliss independence principle are the proposed models for drug combination studies in cancer. The latter is recommended for the prediction of the combination effect if the two drugs target different signaling pathways (Liu *et al.,* 2018). Sorafenib, a tyrosine kinase inhibitor, and paroxetine, a serotonin transporter inhibitor, were considered as two drugs targeting different signaling paths. The 'SynergyFinder' application calculated the synergy scores for the dose-response matrix data based on the

Bliss model, as the two drugs act independently. Figure 1B shows the dose-response matrix where the synergistic effect of the drugs is evident. Reduced cell proliferation was observed after the simultaneous exposure of HepG2 cells to paroxetine (1.9–25 µM) and sorafenib (3.8–50 µM) when compared with each drug alone. Accordingly, treatment with 4.4 µM of paroxetine as a single agent resulted in a 9.5% decrease in cell proliferation. The administration of 8.9 µM of sorafenib caused a 25.6% decrease in cell proliferation. However, when the drugs were applied in combination at the mentioned doses, an inhibition of 50% was obtained. The Bliss energy score and the most synergistic area score were calculated as 15.30 and 33.09, respectively. The 2D and 3D synergy maps highlighted the synergistic dose regions in red, demonstrating a strong synergism (Figures 1C and 1D). A recent study determined the synergistic effect of bis-benzylidine piperidone RA190 and sorafenib in HepG2 cells using SynergyFinder (Soong *et al.,* 2020). Another study used the synergy scoring for the evaluation of sorafenib and sunitinib with multiple-dose combinations in six HCC cell lines. The maximum synergy score in HepG2 cells was reported as 20.18 (Feng *et al*., 2020). The high synergy score in the current study corresponded to strong synergism.

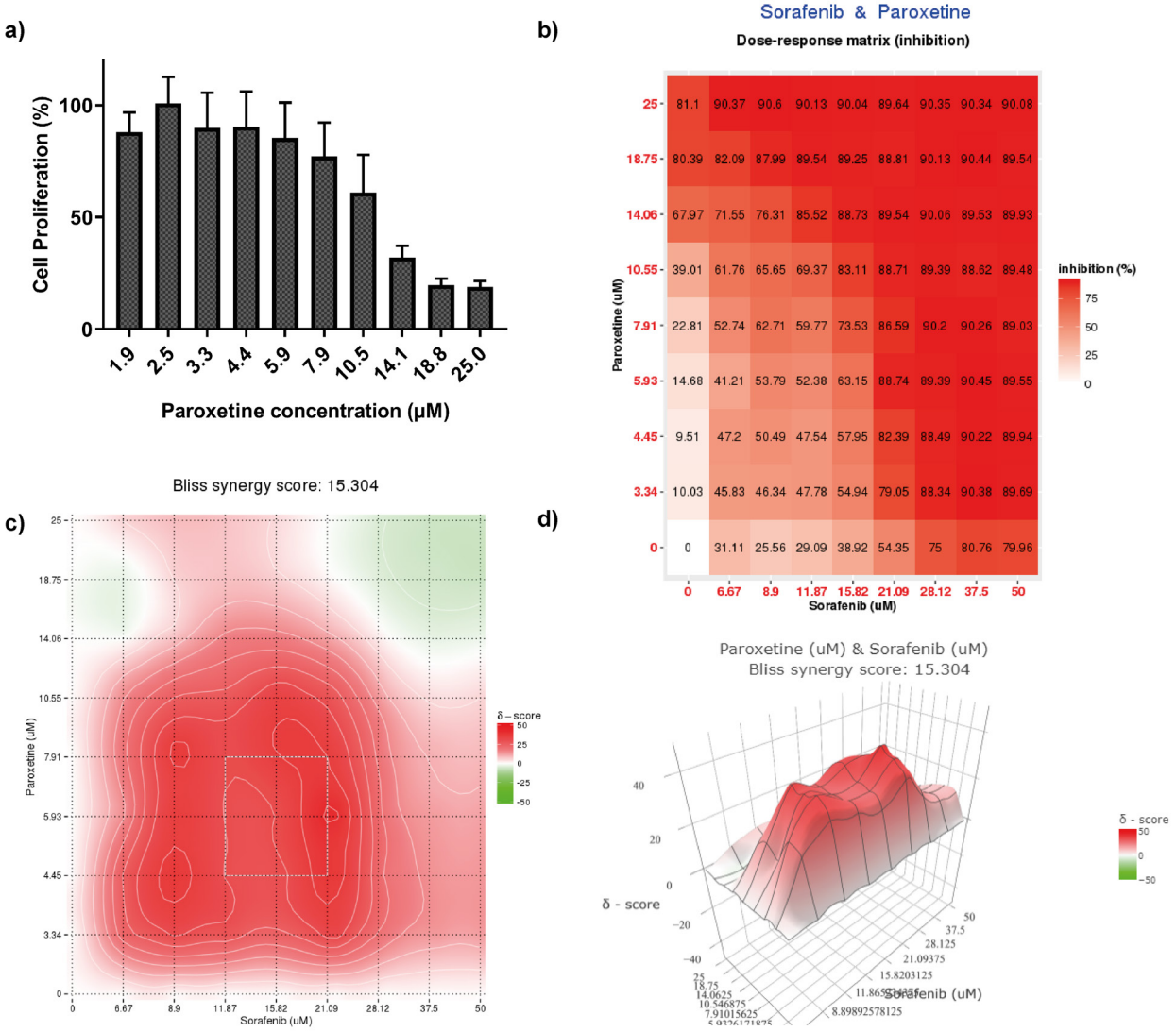


FIGURE 1 - Cytotoxicity of increasing concentrations of paroxetine in HepG2 cells after 24h of treatment (A). Dose-response matrix data demonstrating inhibition of cell proliferation in response to various doses of a paroxetine-sorafenib combination (B). 2D (C) and 3D (D) synergy maps highlighting the synergistic dose regions in red, demonstrating a strong synergism with a Bliss synergy score of 15.304.

Combined effects of paroxetine and sorafenib on apoptosis

The cells were exposed to IC50 doses of the drugs as single agents or in combination to determine whether the treatments would induce apoptosis (Figure 2). The results of the Annexin-V/PI assay were evaluated in comparison to the control group (Figure 2A–2E). The single-agent administration of the HepG2 cells with sorafenib (17.8 μM) or paroxetine (10.2 μM) caused 40% and 43% of the cells to enter apoptosis, respectively. On the other hand, simultaneous combined treatment increased the population of the apoptotic cells to 57% (Figure 2F).

Depression is one of the known adverse effects of sorafenib and paroxetine is the pharmacotherapeutic agent of choice in its treatment. (Bhojani *et al.,* 2008). Several studies have shown that paroxetine also has anti-proliferative and apoptotic effects in a variety of cell lines, such as in osteosarcoma cells (Chou, He, Jan, 2007), OC2 human oral cancer cells (Fang *et al.*, 2011), human lung cancer cells (Rosetti *et al.*, 2006), human T leukemia cells (Amit *et al.*, 2009), human breast cancer cells (Cho *et al.*, 2019), and rat glioma and human neuroblastoma cells (Levkovitz *et al.*, 2005). Two other SSRIs, sertraline and fluoxetine, were also demonstrated to induce apoptosis in vitro (Chen *et al.*, 2014; Khin *et al.*, 2020).

A recent study on human breast cancer MCF-7 cells proposed that paroxetine promotes apoptosis through extracellular Ca2+ and p38 MAPK-dependent ROS generation (Cho *et al.*, 2019). Another study on human colon cancer cells suggested that paroxetine inhibits MET and ERBB3, resulting in the suppression of AKT, ERK, and p38 activation and induction of JNK and caspase-3 pathways (Jang *et al*., 2019). On the other hand, no effect on c-Jun or ERK in malignant T cells was reported (Amit *et al.*, 2009), indicating that the complete mechanism of how paroxetine induces apoptosis is not yet fully understood.

The apoptotic effects of sorafenib on HCC cells have been well established in vitro (Fernando *et al*., 2012; Zhao *et al*., 2013; Garten *et al*., 2019). To the best of our knowledge, the ability of paroxetine to synergically target cancer cells with sorafenib has not been investigated before.

The two-way ANOVA test indicated a significant difference among the groups ($F[6,36] = 4.575$). A significant decrease in the population of live cells and a significant increase in the population of apoptotic cells in comparison to the control group were observed by the co-treatment of paroxetine and sorafenib (34.8 ± 5.0 vs. 58.7 ± 3.0; $P = 0.0071$ and 57.0 ± 4.1 vs. 30.8 ± 1.3; $P = 0.0028$, respectively) (Figure 2F). Accordingly, the combined treatment of paroxetine with sorafenib induced the apoptosis of HepG2 cells.

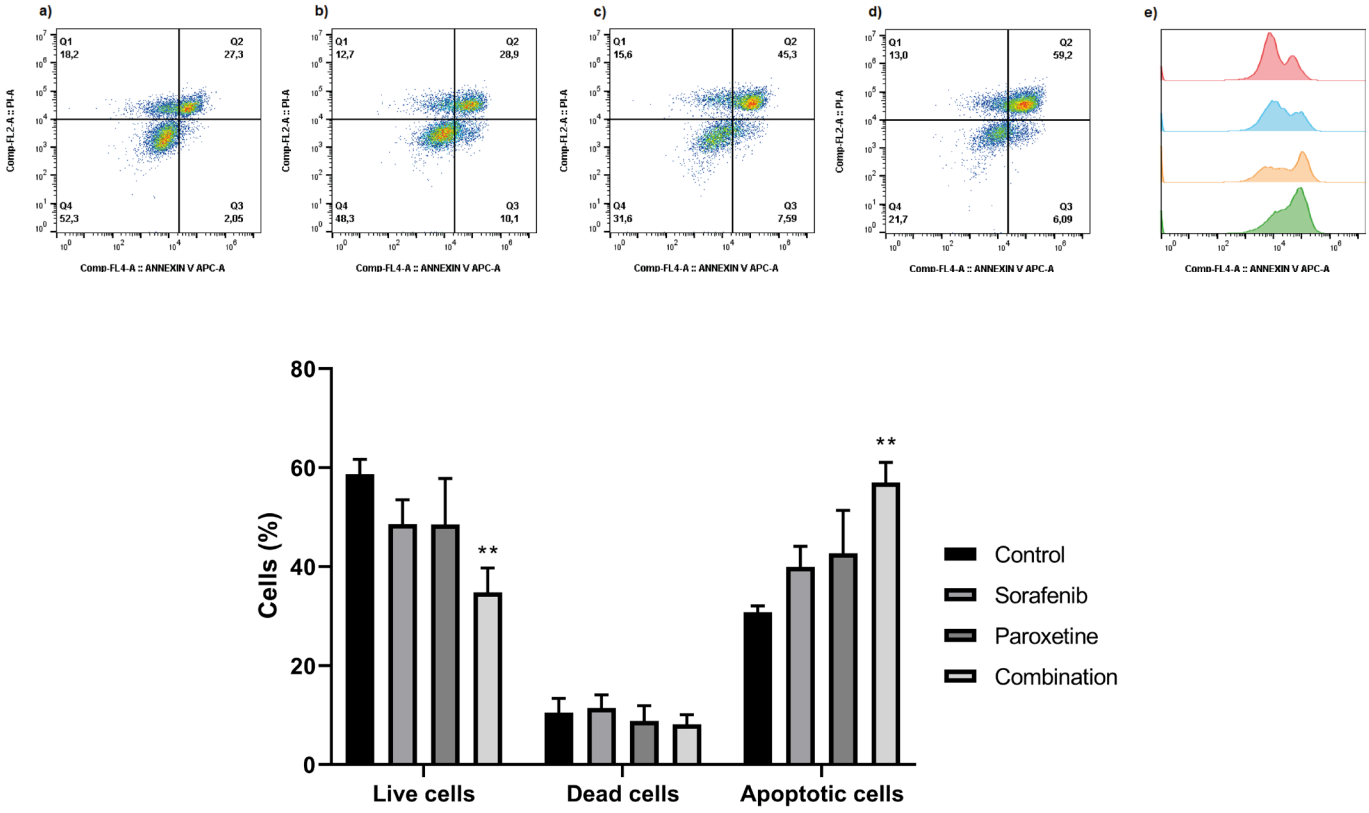


FIGURE 2 - Dot plots demonstrating apoptosis in the cells without any treatment (A), sorafenib-treated (B), paroxetine-treated (C) and co-treated with both drugs (D). Histogram overlay comparing the Annexin V staining in the four groups (E). The bar graph represents the percentages of live cells (Annexin V−/PI−), non-apoptotic dead cells (Annexin V−/PI+), and apoptotic cells that include the cells in apoptosis or those that died by apoptosis (Annexin V+/PI− and Annexin V+/PI+). Two-way ANOVA showed a significant difference among the groups ($F[6,36] = 4.575$). **Significant difference between the combination and control groups at $P < 0.01$.

Combined effects of paroxetine and sorafenib on the cell cycle

As the combined treatment suppressed cell proliferation and induced apoptosis, whether the drugs alter the cell cycle distribution in HepG2 cells was investigated (Figure 3). The DNA contents after treatments were evaluated in the control and drug-treated groups (Figures 3A–3D). The percentage of cells in the G0/G1 phase was shown to be significantly increased after sorafenib treatment as a single agent or in combination with paroxetine. When compared with the control group, the results suggested a cell cycle arrest in G0/G1 phase in the HepG2 cells ($F[6,21] = 6.932$; 61.1 ± 3.4 vs. 43.3 ± 3.5; $P = 0.0042$ and 65.6 ± 3.3 vs. 43.3 ± 3.5; $P = 0.0014$, respectively) (Figure 3E).

G0/G1 phase arrest was demonstrated after sorafenib treatment in HCC cell lines (Abdelmageed *et al.*, 2016; Long *et al.*, 2017). Sorafenib administration, as a single agent or in combination with paroxetine, caused cell cycle arrest in the G0/G1 phase, indicating that sorafenib mainly caused the arrest.

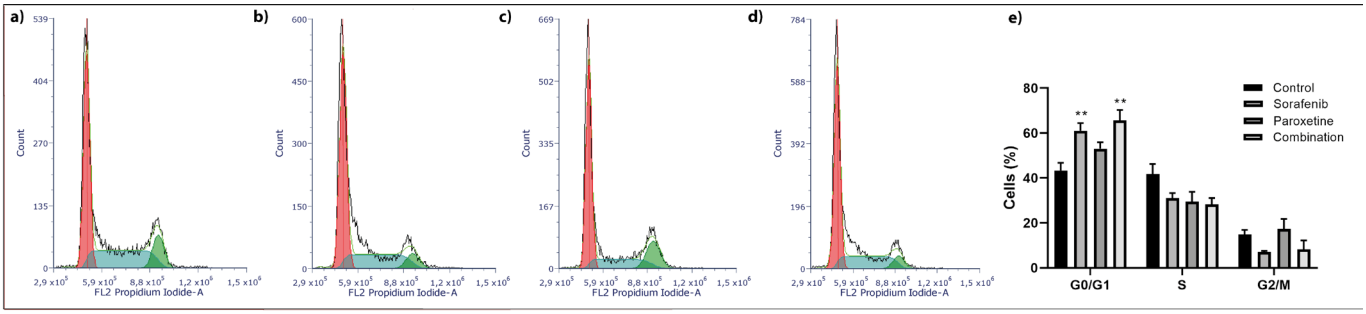
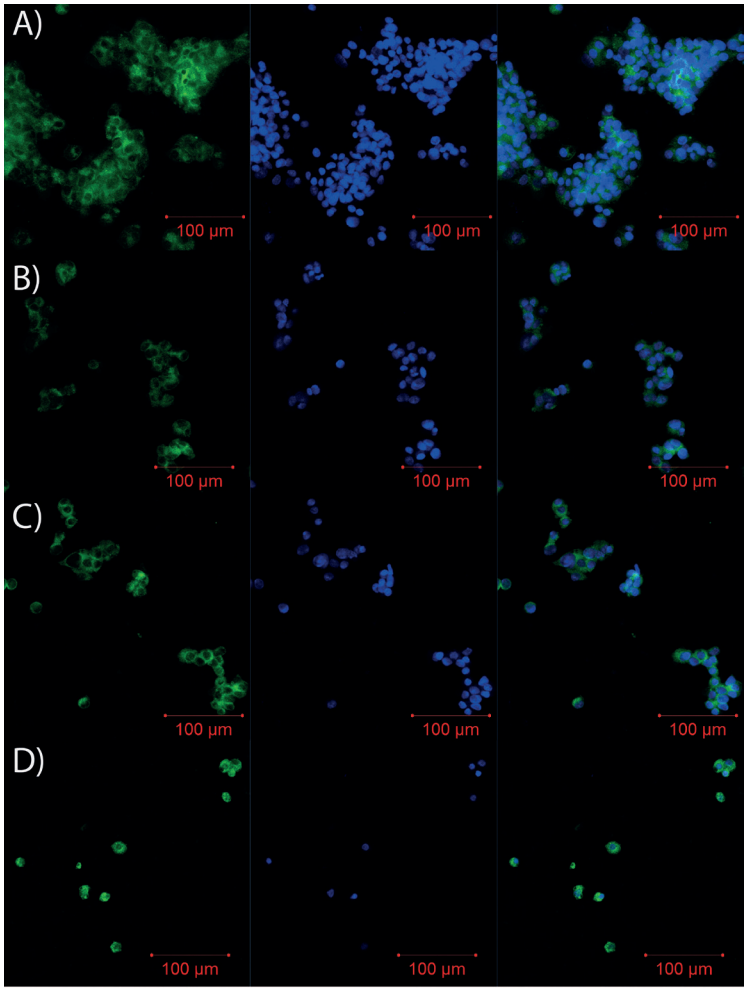


FIGURE 3 - Cell cycle distribution in cells without any treatment (A), sorafenib treated (B), paroxetine treated (C), and co-treated with both drugs (D). The bar graph represents the percentages of cells at the G0/G1, S, and G2/M phases. Two-way ANOVA showed a significant difference among the groups ($F[6,21] = 6.932$). **Significant difference in comparison to the control group at $P < 0.01$.

Evaluation of Bcl-2 expression after combined treatment

The co-treatment of paroxetine with sorafenib resulted in a remarkably reduced cell density and increased staining with the Bcl-2 antibody (Figures 4A–4D). The obtained results showed a significant difference among the groups ($F[3,8 = 18.22]$). The post-hoc analysis demonstrated increased Bcl-2 expression in the cells receiving both paroxetine and sorafenib when compared to the control group (85.9 ± 8.7 vs. 34.8 ± 2.1; $P = 0.0004$). Combined treatment showed a higher Bcl-2 fluorescence signal, also in comparison to the sorafenib-treated cells and paroxetine-treated cells (85.9 ± 8.7 vs. 53.0 ± 2.9; $P = 0.0073$ and (85.9 ± 8.7 vs. 50.7 ± 3.6; $P = 0.0049$, respectively) (Figure 4E). The alterations in the expression of Bcl-2 family proteins were shown to modulate the sorafenib efficacy in HCC, both in vitro and in vivo (Tutusaus *et al.*, 2018). The balance between the pro-apoptotic and pro-survival proteins determines the apoptotic fate of the cell (Singh, Letai, Sarosiek, 2019). In this study, the expression of Bcl-2 was solely investigated. For a comprehensive analysis, this complex mechanism should be examined thoroughly. In a previous study, researchers proposed that the complex nature of apoptotic signaling leads to fractional cell killing, which is more likely with high levels of Bcl-2 (Skommer, Brittain, Raychaudhuri, 2010). This might explain the lower cell density and the high Bcl-2 expression of these remaining cells observed under confocal microscope after coadministration of the drugs.



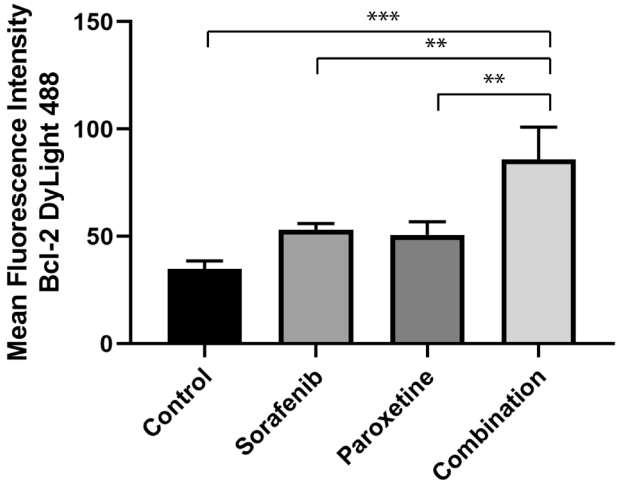


FIGURE 4 - Immunofluorescence detection of anti-apoptotic Bcl-2 in cells without any treatment (A), sorafenib treated (B), paroxetine treated (C), and co-treated with both drugs (D). After 24 h of incubation, the cells were labeled with the Bcl-2 antibody. IgG DyLight®488 was used as the secondary antibody. The nuclei were counterstained with Hoechst 33258. Bcl-2 fluorescence intensity was plotted (E). One-way ANOVA showed a significant difference among the groups ($F[3,8 = 18.22]$) **Significant difference in comparison to the sorafenib or paroxetine treated cells at $P < 0.01$. ***Significant difference in comparison to the control group at $P < 0.001$.

CONCLUSION

Coadministered paroxetine potentiated the anti-proliferative and apoptotic effects of sorafenib. Despite the advantages of the combination of paroxetine and sorafenib treatment, adverse effects due to sorafenib might still occur. Moreover, further clinical evidence is required to determine the balance of benefit and harm of paroxetine in the absence of depression. This study may provide a starting point for repurposing paroxetine as a new indication for HCC treatment and a potential therapeutic strategy for patients with HCC.

ACKNOWLEDGEMENTS

The authors would like to thank Assoc. Prof. David T. Thomas, MD. (Maltepe University School of Medicine, Department of Medical Education) for editing the manuscript. This study was funded by the Maltepe University Research Project Council (Grant date 14.02.2018).

REFERENCES

Abdelmageed MM, El-Naga RN, El-Demerdash E, Elmazar MM. Indole-3- carbinol enhances sorafenib cytotoxicity in hepatocellular carcinoma cells: A mechanistic study. Sci Rep. 2016;6:32733.

Amit BH, Gil-Ad I, Taler M, Bar M, Zolokov A, Weizman A. Proapoptotic and chemosensitizing effects of selective serotonin reuptake inhibitors on T cell lymphoma/leukemia (Jurkat) in vitro. Eur Neuropsychopharmacol. 2009;19(10):726-34.

Bangaru S, Marrero JS, Singal AG. Review article: new therapeutic interventions for advanced hepatocellular carcinoma. Aliment Pharmacol Ther. 2020;51(1):78-89.

Bhojani N, Jeldres C, Patard JJ, Perrotte P, Suardi N, Hutterer G, et al. Toxicities associated with the administration of sorafenib, sunitinib, and temsirolimus and their management in patients with metastatic renal cell carcinoma. Eur Urol. 2008;53(5):917-30.

Bliss CI. The toxicity of poisons applied jointly. Ann Appl Biol. 1939;26(3):585-615.

Bourin M, Chue P, Guillon Y. Paroxetine: a review. CNS Drug Rev. 2001;7(1):25-47.

Cabral L, Tiribelli C, Sukowati C. Sorafenib resistance in hepatocellular carcinoma: the relevance of genetic heterogeneity. Cancers. 2020;12(6):1576.

Chan HL, Chiu WC, Chen VC, Huang KY, Wang TN, Lee Y, et al. SSRIs associated with decreased risk of hepatocellular carcinoma: A population-based case-control study. Psycho-Oncology. 2018;27(1):187-92.

Chen S, Xuan J, Wan L, Lin H, Couch L, Mei N, et al. Sertraline, an antidepressant, induces apoptosis in hepatic cells through the mitogen-activated protein kinase pathway. Toxicol Sci. 2014;137(2):404-15.

Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol. 2009;10(1):25-34.

Cho YW, Kim EJ, Nyiramana MM, Shin EJ, Jin H, Ryu JH, et al. Paroxetine induces apoptosis of human breast cancer MCF-7 Cells through Ca(2+)-and p38 MAP kinase-dependent ROS generation. Cancers (Basel). 2019;11(1):64.

Chou CT, He S, Jan CR. Paroxetine-induced apoptosis in human osteosarcoma cells: activation of p38 MAP kinase and caspase-3 pathways without involvement of [Ca2+]i elevation. Toxicol Appl Pharmacol. 2007;218(3):265-73.

Davis BA, Nagarajan A, Forrest LR, Singh SK. Mechanism of paroxetine (Paxil) inhibition of the serotonin transporter. Sci Rep. 2016;6:23789.

Fang YC, Chou CT, Pan CC, Hsieh YD, Liang WZ, Chao D, et al. Paroxetine-induced Ca2+ movement and death in OC2 human oral cancer cells. Chin J Physiol. 2011;54(5):310-7.

Feng F, Zhang Z, Ding G, Hui L, Li Y, Li H. Personalized anti-cancer drug combination prediction by an Integrated Multi-level Network. BioRxiv 2020;2020.05.12.092239.

Fernando J, Sancho P, Fernandez-Rodriguez CM, Lledo JL, Caja L, Campbell JS, et al. Sorafenib sensitizes hepatocellular carcinoma cells to physiological apoptotic stimuli. J Cell Physiol. 2012;227(4):1319-25.

Garten A, Grohmann T, Kluckova K, Lavery GG, Kiess W, Penke M. Sorafenib-Induced Apoptosis in Hepatocellular Carcinoma Is Reversed by SIRT1. Int J Mol Sci. 2019;20(16):4048.

Gauthier A, Ho M. Role of sorafenib in the treatment of advanced hepatocellular carcinoma: An update. Hepatol Res. 2013;43(2):147-54.

Ghouri YA, Mian I, Rowe JH. Review of hepatocellular carcinoma: Epidemiology, etiology, and carcinogenesis. J Carcinog. 2017;16:1.

Hampton T. Cancer drug trials show modest benefit: drugs target liver, gastric, head and neck cancers. J Am Med Assoc. 2007;298(3):273-5.

Huang CC, Chen HY, Chang RH, Liao PA, Lien HH, Hung CS, et al. A real-life experience of sorafenib treatment for patients with advanced hepatocellular carcinoma: a retrospective analysis at Cathay General Hospital, 2007-2015. Drug Des Devel Ther. 2019;13:397-404.

Ianevski A, He L, Aittokallio T, Tang J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. Bioinformatics. 2017;33(15):2413-15.

Jang WJ, Jung SK, Vo TTL, Jeong CH. Anticancer activity of paroxetine in human colon cancer cells: Involvement of MET and ERBB3. J Cell Mol Med. 2019;23(2):1106-15.

Khin PP, Po WW, Thein W, Sohn UD. Apoptotic effect of fluoxetine through the endoplasmic reticulum stress pathway in the human gastric cancer cell line AGS. Naunyn-Schmiedeberg's Arch Pharmacol. 2020;393(4):537-49.

Kuwahara J, Yamada T, Egashira N, Ueda M, Zukeyama N, Ushio S, et al. Comparison of the anti-tumor effects of selective serotonin reuptake inhibitors as well as serotonin and norepinephrine reuptake inhibitors in human hepatocellular carcinoma cells. Biol Pharm Bull. 2015;38(9):1410-4.

Levkovitz Y, Gil-Ad I, Zeldich E, Dayag M, Weizman A. Differential induction of apoptosis by antidepressants in glioma and neuroblastoma cell lines: evidence for p-c-Jun, cytochrome c, and caspase-3 involvement. J Mol Neurosci. 2005;27(1):29-42.

Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021;7(1):6.

Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359(4):378-90.

Liu Q, Yin X, Languino LR, Altieri DC. Evaluation of drug combination effect using a Bliss independence dose-response surface model. Stat Biopharm Res. 2018;10(2):112-122.

Long F, Dong C, Jiang K, Xu Y, Chi X, Sun D, et al. Melatonin enhances the anti-tumor effect of sorafenib via AKT/p27-mediated cell cycle arrest in hepatocarcinoma cell lines. RSC Advances. 2017;7(34):21342-51.

Longo L, de Freitas LBR, Santos D, Grivicich I, Alvares-da-Silva MR. Sorafenib for Advanced Hepatocellular Carcinoma: A Real-Life Experience. Dig Dis. 2018;36(5):377-84.

Ozunal ZG, Donmez Cakil Y, Isan H, Saglam E, Aktas RG. Sertraline in combination with sorafenib: A promising pharmacotherapy to target both depressive disorders and hepatocellular cancer. Biol Futur. 2019;70(4):341-48.

Rosetti M, Frasnelli M, Tesei A, Zoli W, and Conti M. Cytotoxicity of different selective serotonin reuptake inhibitors (SSRIs) against cancer cells. J Exp Ther Oncol. 2006;6(1):23-9.

Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. Nat Rev Mol Cell Biol. 2019;20(3):175-193.

Skommer J, Brittain T, Raychaudhuri S. Bcl-2 inhibits apoptosis by increasing the time-to-death and intrinsic cell-to-cell variations in the mitochondrial pathway of cell death. Apoptosis. 2010;15(10):1223-33.

Smith HR. Depression in cancer patients: Pathogenesis, implications and treatment (Review). Oncol Letters. 2015;9(4):1509-14.

Soong RS, Anchoori RK, Roden RBS, Cho RL, Chen YC, Chieh S, et al. Bis-benzylidine Piperidone RA190 treatment of hepatocellular carcinoma via binding RPN13 and inhibiting NF-κB signaling. BMC Cancer. 2020;20(1):386.

Tutusaus A, Stefanovic M, Boix L, Cucarull B, Zamora A, Blasco L, et al. Antiapoptotic BCL-2 proteins determine sorafenib/regorafenib resistance and BH3-mimetic efficacy in hepatocellular carcinoma. Oncotarget. 2018;9(24):16701-16717.

Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380(15):1450-62.

Zhao X, Tian C, Puszyk WM, Ogunwobi OO, Cao M, Wang T, et al. OPA1 downregulation is involved in sorafenib-induced apoptosis in hepatocellular carcinoma. Lab Invest. 2013;93(1):8-19.

Zhu YJ, Zheng B, Wang HY, Chen L. New knowledge of the mechanisms of sorafenib resistance in liver cancer. Acta Pharmacol Sin. 2017;38(5):614-22.

Received for publication on 23rd December 2020
Accepted for publication on 08th June 2021