Celal Bayar University Journal of Science

Boric Acid Treatment Strengthens the Cytotoxic Effect of Sorafenib on Triple Negative Breast Cancer Cell Lines

Erkan Kahraman[1*], Erdem Göker[2]

[1]Ege University, Atatürk Health Care Vocational School, Bornova, İzmir, Türkiye
[2]Ege University, Faculty of Medicine Department of Internal Medicine, Bornova, İzmir, Türkiye
*erkan.kahraman@ege.edu.tr
* Orcid No: 0000-0003-0051-416X

Received: 8 November 2022
Accepted: 12 June 2023
DOI: 10.18466/cbayarfbe.1201068

Abstract

In recent years, it has been demonstrated that combinational therapies have shown promising results in the treatment of triple negative breast cancer. However, the effect of the sequential combination of sorafenib with boric acid on cell viability in triple negative breast cancer cell lines is unknown. Thus, the present study aims to investigate the effects of sequential treatment of boric acid and sorafenib on cell viability in triple negative breast cancer cell lines. MDA-MB-231 cells were used in our study. Sorafenib was treated to the cells at a dose range of 0.5-16µM, and boric acid at 1-160mM. Changes in cell viability were determined using by MTT analysis at 24,48 and 72 hours. Cell viability decreased statistically significantly at 4µM and above doses of sorafenib, and 5mM and above doses of boric acid ($p<0.05$). IC50 values of boric acid were calculated as 34mM, 26mM and 1mM at hours 24,48 and 72, respectively. Alone treatment of sorafenib at 8µM and 16µM doses reduced cell viability up to 80% and 61%, respectively. On the other hand, 15mM boric acid treatment followed by sorafenib treatment at 8µM and 16µM doses decreased cell viability up to 64% and 44%, respectively ($p<0.05$). Also, it was observed that boric acid treatment followed by sorafenib treatment caused MDA-MB-231 cells to diverge from their ancestral morphology, resulting in an unhealthier appearance. Our results suggest that a sequential treatment of boric acid followed by sorafenib strengthens the cytotoxic effect of sorafenib on triple negative breast cancer cell lines.

Keywords: Boric Acid, Boron, Sorafenib, Triple negative breast cancer

1. Introduction

Breast cancer ranks as the first most common cancer in worldwide [1]. The disease can occur in different sites of the breast, including the lobules, canals and connective tissues. Breast cancer exhibits various physiological characteristics specific to the tissue in which it originates, and the different clinical outcomes that can occur have led to the creation of various subgroups [2]. Triple-negative breast cancer (TNBC) is more aggressive and is more prone to recurrence than other subtypes [3]. Among the breast cancer subtypes, TNBC cancer has limited therapeutic options due to the lack of well-established molecular targets for targeted therapy. Accordingly, the development of new treatment strategies is of critical importance [4]. While classic chemotherapeutic approaches are still valid, studies have identified various combination regimens as potentially effective strategies [5].

Sorafenib, an orally administered multi-kinase inhibitor, has been used effectively for the treatment of unresectable hepatocellular carcinoma (HCC) since 2008 [6]. Sorafenib is able to block cellular proliferation by inhibiting the activity of kinases involved in the extracellular signal-regulated kinase Raf-1, B-Raf and Ras/Raf/MEK/ERK signaling pathway, and also suppresses angiogenesis by inhibiting the hepatocyte factor receptor (c-Kit), Fmes-like tyrosine kinase (FLT-3), VEGFR-2 and VEGFR-3, PDGFR-β and other kinases [7]. Since its initial introduction into practice, it has been included as part of effective therapeutic approaches to the treatment of HCC [8], and has also been reported to inhibit the pathogenesis of breast cancer [9]. The growth-inhibiting effects and favorable toxicity profile of sorafenib in preclinical models have revealed potential benefits in breast cancer [10].

Boron is placed in group 13 of the periodic table, and it is only non-metal element in this group. It is not present

in its elemental form in nature. Boron is found in nature as a component of boric acid (BA), kernite, borax, ulexite and colemanite in nature [11]. In the human body, inorganic borate compounds are found in the BA form.[12]. It has been suggested that BA can play a beneficial role in anti-cancer processes in specific cancer types, and has shown promise in this regard [13]. Epidemiological studies have also shown that the addition of BA to the diet decreases the risk of the development of various cancers, including those of the prostate and the lung [14]. A number of *in vitro* studies have also reported its possible anti-cancer properties in various cancer cell lines, and it has been demonstrated that BA induces apoptosis and suppresses cellular proliferation in breast cancer cells [15-17].

The present study, performed in light of the above data, investigates to effect of sequential treatment of the FDA-approved drug sorafenib and BA, which is reported to possess anti-cancer properties, on cell viability in TNBC cells lines.

2. Materials and Methods

2.1. Cell Culture

All procedures in this study were performed in vitro in commercially purchased human Triple Negative (ER-/PR-/HER-) MDA-MB-231 cell lines. TNBC cell lines were cultured in MEM medium (Capricorn, Cat. No:MEM-A) supplemented with 10% Fetal Bovine Serum (Biol. Ind. Cat. No:01-121-1A), 100 µg/ml Penicillin / Streptomycin (Biol. Ind. Cat. No:03-031-1B), 1% MEM non-essential amino acid solution (Biol. Ind. Cat. No:01-340-1B) at 37 °C with 5% CO_2 in a humidified atmosphere.

2.2. Cell viability assay

A MTT assay was carried out for the determination of cell viability of TNBC cells. Briefly, cells were seeded to 96 well plates at 5000 cell / well concentrations and incubated for 24 hours. After the incubation period, the cell culture media were discarded. Sorafenib (0.5, 1, 2, 4, 8, 16 µM) and BA (1, 5, 10, 15, 20, 30, 40, 80, 160 mM) contained fresh media was treated to cells. End of the treatment time, 15µl MTT (5mg/ml, Sigma, Cat. No: M5655) solution was added to each well and incubated 4 hours. Mediums were removed and 100µl DMSO (Merck, Cat. No:116743) added to wells. Plates were read at 570nm wavelength using by a microplate reader. Viability of TNBC cells were calculated according to the formula below.
Viability (%)= (OD, treated group- blank)-(OD, untreated group- blank)x100

2.3. Sequential treatment of BA and sorafenib

For sequential treatment, BA was used at 15 mM dose, and sorafenib 8 and 16 mM doses. Cells were seeded to 96 well plates at 5000 cells/well concentration and cultured for 24 hours. Cell culture mediums were changed with fresh medium containing BA at 15mM dose. After 24 hours, mediums were replaced with fresh medium containing sorafenib at 8 and 16 µM doses and cells were incubated for 72 hours. Effect of sequential treatment on cell viability were determined using by a MTT analysis. Also, cellular morphological changes were examined under light microscope.

2.4. Statistical analysis

All statistical analyses were performed using by Graph Pad Prism 8.4 statistical software. One-way ANOVA was used for comparison of multiple groups and post hoc Tukey's test was used between groups. To determine the significance level of the difference between the means of the two groups, independent sample t-test was performed. Dose response inhibition analysis was carried out for calculation of IC50 values. All assays were made at least three times with three replicates. A p-value of <0.05 was taken as statistically significant.

3. Results

3.1. Effect of Sorafenib on Cell Viability

The study investigated the effects of sorafenib up to a dose of 16 µM on the viability of cells at 24,48 and 72 hours after treatment, and found it to have no effect on cell viability in doses up to 2 µM, while doses of 4 µM or greater significantly reduced cell viability. Also, it was founded that sorafenib reduced the viability of cells in a dose- and time-dependent manner at other doses and time points. (Figure 1). According to the results of cell viability analysis, sorafenib doses of 8 and 16 µM were determined as appropriate for use in sequential treatment.

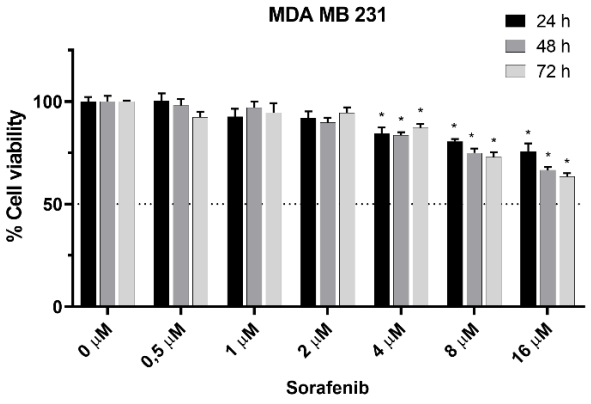

Figure 1. Effect of sorafenib on cell viability. Error bars shows ± SD (n=3). * Asterisk shows p<0.05.

Celal Bayar University Journal of Science
Volume 19, Issue 2, 2023, p 137-141
Doi: 10.18466/cbayarfbe.1201068

3.2. Effect of BA on Cell Viability

The effects of BA up to doses of 160 µM were investigated on the viability of cells at 24,48 and 72 hours, and it was observed that BA at doses of 5 mM and greater significantly reduced cell viability (Figure 2). The IC50 values of BA at 24, 48 and 72 hours were calculated as 34, 26 and 15 mM, respectively (Figure 3). A boric dose of 15 mM was determined for use in sequential treatment, and was found to reduce the viability of cells in a dose and time dependent manner.

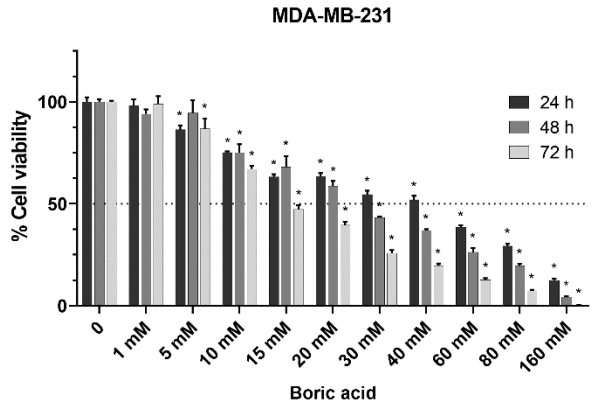

Figure 2. Effect of BA on cell viability. Error bars shows ± SD (n=3). * Asterisk shows p<0.05.

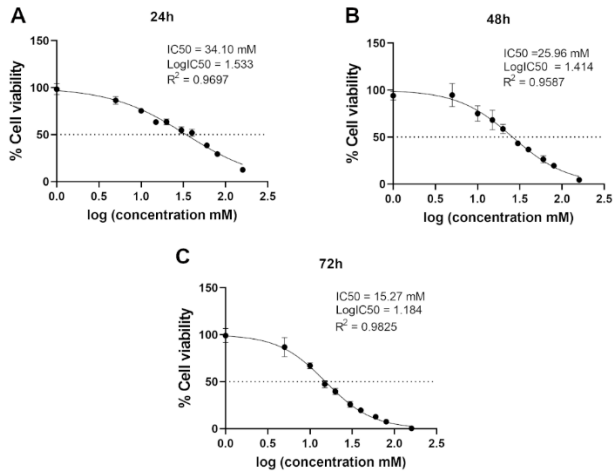

Figure 3. IC50 values of BA. Changes in viability were determined by MTT analysis. IC50 values of BA were calculated at 24 (A), 48 (B) and 72 (C) hours. Error bars shows ± SD (n=3). * Asterisk shows p<0.05.

3.3. Effect of sequential treatment of BA and sorafenib on cell viability.

The treatment of BA alone reduced cell viability by 88%, and the treatment of sorafenib alone at doses of 8 µM and 16 µM reduced cell viability by 80% and 44%, respectively.

The treatment of sorafenib at doses of 8 µM and 16 µM after treatment with BA for 24 hours reduced cell viability by 64% and 44%, respectively. The reduction in cell viability following both sequential treatment was statistically significant when compared to the single treatment of either BA or sorafenib ($p<0.05$) (Figure 4). On the other hand, no changes were observed in cell morphology after the BA treatment at the specified doses. In cell lines treated only with sorafenib, the cellular morphology changed, cells lost their cell-to-cell connections, the cytoplasm decreased, and the cell morphology transformed from the ancestral morphology and took on an unhealthy appearance. This effect was even more prominent in combinational doses (Figure 5). In conclusion, sorafenib treatment for 72 hours following BA treatment for 24 hours was noted to strengthen the anti-cancer effect of sorafenib in TNBC cell lines.

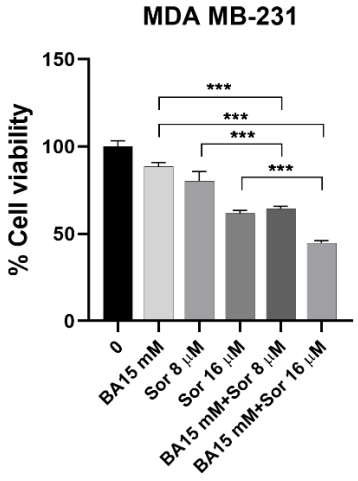

Figure 4. Effect of sequential treatment of BA and sorafenib on cell viability. Following 24 hours BA treatment, sorafenib was treated to cells for 72 hours. Error bars shows ± SD (n=3). * Asterisk shows p<0.05.

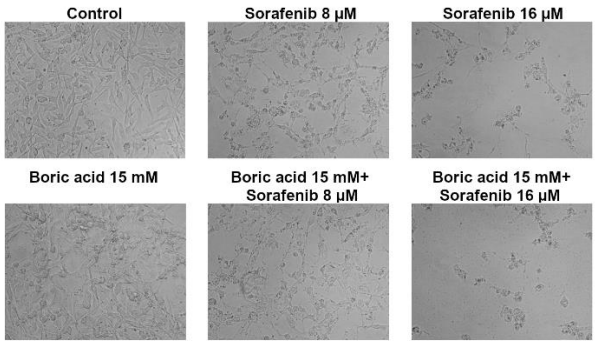

Figure 5. Effect of sequential treatment of BA and sorafenib on cellular morphology. Following 24 hours BA treatment, sorafenib was treated to MDA-MB-231 cell lines for 72 hours. Cellular morphological changes were examined under light microscope.

4. Discussion

TNBC is a heterogeneous malignancy that exhibits distinct characteristics in terms of its natural course and response to therapy. Patients do not benefit from hormonal therapies due to the loss of such target receptors as the ER, PR and HER-2. For this reason, chemotherapy still remains the optimum treatment [18], despite the potential poor response, increased toxicity and drug resistance, which may lead to poor outcomes [19]. Although surgery and chemotherapy, alone or in combination, may seem to be the sole treatment modalities available, recent studies have reported promising effects of combination therapies in the treatment of TNBC, suggesting them as a good therapeutic option [20]. The efficacy of sorafenib has been investigated when used in combination with other drugs for the treatment of breast cancer [10].

Sorafenib has been investigated in numerous clinical trials involving patients with breast cancer, and has often been reported to be well-tolerated in patients with metastatic disease and in those with earlier-stage breast cancer [21]. The possible mechanisms underlying its anti-cancer properties have been linked to its inhibition of tumor progression and growth, inhibition of angiogenesis and metastasis. However, aside from in clinical trials, sorafenib is not currently part of routine treatment approaches to breast cancer [22]

Sorafenib has been shown to reduce cell viability at doses of 1 µM in Mahlavu cell lines [15], at doses of 4 µM in HepG2 and HuH-7 cell lines [23], at doses of 1 µM in LNCAP cell lines, and at doses of 0.5 µM and greater in PC3 cell lines [24]. Cytotoxic effects have been shown to occur at doses of 10 µM and greater in ER positive / PR positive breast cancer cell lines [25]. In the present study, sorafenib reduced cell viability in TNBC cell lines, at doses of 4 µM and greater at 24, 48 and 72 hours. Consequently, the present study found that sorafenib reduced the viability of TNBC cells in a dose and time dependent manner.

There have been many experimental studies reporting the anti-cancer properties of BA and its promise as a cancer treatment. Studies of cultured cancer cells have reported an IC50 value at 24 hours of 30 mM in Mahlavu cell lines, 20 mM in HuH-7 cell lines [15], 17 mM in U87-MG cell lines [26] and 10.7 mM in DU-145 cell lines [27]. In the present study, the IC50 value at 24 hours was 34 mM in TNBC cells, while the IC50 values at 48 and 72 hours were 26 mM and 15 mM, respectively. It was observed, however, that the dose-reducing cell viability was 5 mM and greater, it was determined that the dose that reducing cell viability was 5 mM and greater. Consequently, it was concluded that BA reduces the viability of TNBC cell lines in a dose and time dependent manner.

Although single-agent treatment regimens have shown favorable results on cell morphology and in preclinical models, clinical studies have failed to produce promising results in aggressive TNBC due to its heterogeneous nature and the development of drug resistance [28]. For this reason, combined drug therapies are gaining popularity and have proven to be effective in clinical trials in improving complete pathologic response (PCR), progression-free survival (PFS) and overall survival (OS). Almost 80% of the currently ongoing clinical trials investigating new therapeutic strategies for the treatment of TNBC are exploring the efficacy of various drug combinations [5]. Sorafenib is one such drug being investigated, for use in combination with such agents as paclitaxel and capecitabine, all of which have produced promising results in the treatment of TNBC [29, 30]. The present study demonstrates that sorafenib used in combination with BA significantly reduces cell viability when compared to the treatment of sorafenib alone or the treatment of BA alone. The study found that the combined regimen also affects cell morphology more profoundly than the single treatment of the agents, resulting in an unhealthy appearance in the cells.

5. Conclusion

The sequential treatment of BA and sorafenib as a combinational regimen strengthens the anti-cancer efficacy of sorafenib in TNBC cell lines. The elucidation of the molecular mechanisms underlying the effect of sorafenib and BA combination would aid in the establishment of the optimal doses and sequential administration of each agent, and may provide to significant strides in the management of this difficult-to-treat condition.

Acknowledgement

This work was supported by AKOD (Academic Oncology Association from Türkiye)

Author's Contributions

Erkan Kahraman:
Erdem Göker:

Ethics

There are no ethical issues after the publication of this manuscript.

References

[1]. Bray F, Ferlay J, Soerjomataram I, et al. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*; 68: 394-424.

[2]. Weigelt B, Geyer FC, Reis-Filho JS. 2010. Histological types of breast cancer: How special are they? *Molecular Oncology*; 4: 192-208.

[3]. Garrido-Castro AC, Lin NU, Polyak K. 2019. Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for TreatmentHeterogeneity of Triple-Negative Breast Cancer. *Cancer discovery*; 9: 176-198.

[4]. Yin L, Duan J-J, Bian X-W, et al. 2020. Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Research*; 22: 61.

[5]. Chalakur-Ramireddy Naveen KR, Pakala Suresh B. 2018. Combined drug therapeutic strategies for the effective treatment of Triple Negative Breast Cancer. *Bioscience Reports*; 38: BSR20171357.

[6]. Llovet JM, Ricci S, Mazzaferro V, et al. 2008. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*; 359: 378-390.

[7]. Wilhelm SM, Carter C, Tang L, et al. 2004. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*; 64: 7099-7109.

[8]. Marisi G, Cucchetti A, Ulivi P, et al. 2018. Ten years of sorafenib in hepatocellular carcinoma: Are there any predictive and/or prognostic markers? *World J Gastroenterol*; 24: 4152-4163.

[9]. Moreno-Aspitia A. 2010. Clinical overview of sorafenib in breast cancer. *Future Oncology*; 6: 655-663.

[10]. Bianchi G, Loibl S, Zamagni C, et al. 2009. Phase II multicenter, uncontrolled trial of sorafenib in patients with metastatic breast cancer. *Anti-Cancer Drugs*; 20: 616-624.

[11]. Kot FS. 2009. Boron sources, speciation and its potential impact on health. *Reviews in Environmental Science and Bio/Technology*; 8: 3-28.

[12]. Smallwood C. International Programme on Chemical Safety Environmental Health Criteria 204 Boron; WHO: Geneva, 1998;

[13]. Barranco W, Eckhert C. 2006. Cellular changes in boric acid-treated DU-145 prostate cancer cells. *British journal of cancer*; 94: 884-890.

[14]. Cui Y, Winton MI, Zhang Z-F, et al. 2004. Dietary boron intake and prostate cancer risk. *Oncol Rep*; 11: 887-892.

[15]. Kahraman E, Göker E. 2022. Boric acid exert anti-cancer effect in poorly differentiated hepatocellular carcinoma cells via inhibition of AKT signaling pathway. *Journal of Trace Elements in Medicine and Biology*; 73: 127043.

[16]. Kahraman E, Gürhan İD, Korkmaz M. 2013. Investigation of possible genotoxic and cytotoxic effects of differential boron compounds in ccl 62 (hela contaminant) human amniotic ephitelial cell line. *Medicine Science*; 2: 454-468.

[17]. Meacham SL, Hall C, Tharkar S, et al. Boric acid induces apoptosis in some breast cancer cell lines. In.: Wiley Online Library; 2006.

[18]. Lebert JM, Lester R, Powell E, et al. 2018. Advances in the Systemic Treatment of Triple-Negative Breast Cancer. *Current Oncology*; 25: 142-150.

[19]. Nedeljković M, Damjanović A. 2019. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer—How We Can Rise to the Challenge. *Cells*; 8: 957.

[20]. Wahba HA, El-Hadaad HA. 2015. Current approaches in treatment of triple-negative breast cancer. *Cancer Biol Med*; 12: 106-116.

[21]. Zafrakas M, Papasozomenou P, Emmanouilides C. 2016. Sorafenib in breast cancer treatment: A systematic review and overview of clinical trials. *World J Clin Oncol*; 7: 331-336.

[22]. Bronte G, Andreis D, Bravaccini S, et al. 2017. Sorafenib for the treatment of breast cancer. *Expert Opinion on Pharmacotherapy*; 18: 621-630.

[23]. Wei J-c, Meng F-d, Qu K, et al. 2015. Sorafenib inhibits proliferation and invasion of human hepatocellular carcinoma cells via up-regulation of p53 and suppressing FoxM1. *Acta Pharmacologica Sinica*; 36: 241-251.

[24]. Oh SJ, Erb HH, Hobisch A, et al. 2012. Sorafenib decreases proliferation and induces apoptosis of prostate cancer cells by inhibition of the androgen receptor and Akt signaling pathways. *Endocr Relat Cancer*; 19: 305-319.

[25]. Dattachoudhury S, Sharma R, Kumar A, et al. 2020. Sorafenib inhibits proliferation, migration and invasion of breast cancer cells. *Oncology*; 98: 478-486.

[26]. Aydin HE, Koldemir-Gündüz M, Kizmazoglu C, et al. 2021. Cytotoxic effect of boron application on glioblastoma cells. *Turk Neurosurg*; 31: 206-210.

[27]. Hacioglu C, Kar F, Kacar S, et al. 2020. High Concentrations of Boric Acid Trigger Concentration-Dependent Oxidative Stress, Apoptotic Pathways and Morphological Alterations in DU-145 Human Prostate Cancer Cell Line. *Biological Trace Element Research*; 193: 400-409.

[28]. Lee A, Djamgoz MBA. 2018. Triple negative breast cancer: Emerging therapeutic modalities and novel combination therapies. *Cancer Treatment Reviews*; 62: 110-122.

[29]. Gradishar WJ, Kaklamani V, Sahoo TP, et al. 2013. A double-blind, randomised, placebo-controlled, phase 2b study evaluating sorafenib in combination with paclitaxel as a first-line therapy in patients with HER2-negative advanced breast cancer. *European Journal of Cancer*; 49: 312-322.

[30]. Baselga J, Segalla JGM, Roché H, et al. 2012. Sorafenib in Combination With Capecitabine: An Oral Regimen for Patients With HER2-Negative Locally Advanced or Metastatic Breast Cancer. *Journal of Clinical Oncology*; 30: 1484-1491.