IJMPR 2020, 4(1), 18-22

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article

SJIF Impact Factor: 5.273

INVESTIGATION OF APOPTOTIC AND ANTIANGIOGENIC EFFECTS OF BORON IN MCF-7 CELLS

Ali Osman Albayrak[1]*, Funda Karabağ Çoban[2], Mehmet Emrah Şelli[2] and Hande Aytuğ[1]

[1]Molecular Biology and Genetics A.D, İnstitute of Sciences, Usak University Usak 64200, Turkey.
[2]Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Usak University, Usak 64200, Turkey.

Received on: 30/11/2019
Revised on: 20/12/2019
Accepted on: 10//12/2020

***Corresponding Author**
Ali Osman Albayrak
Molecular Biology and Genetics A.D, İnstitute of Sciences, Usak University Usak 64200, Turkey.

ABSTRACT

Breast cancer is the most common type of cancer among women and ranks second among the causes of female death in the world. In order to find a solution to breast cancer, different studies are being conducted for the treatment and the effects of different drugs and substances on this disease are intensively investigated. Boric acid has been shown to control the proliferation of certain types of cancer cells. In the study, different concentrations of boric acid were applied on MCF-7 cell line to determine IC50 values of boric acid and its antiproliferative effect was determined using Cell Counting Kit-8 (CCK8) ELISA kit at 24, 48 and 72 hours. According to the results, other analyzes were performed over the 48 hour incubation period. After the IC50 values of boric acid at 48 hours were determined, Poly ADP-ribose polymerase (PARP) kit was used to determine apoptotic effects for boric acid. Cell lysates were obtained after the application of defined IC50 doses to the cells for determination of biochemical parameters. The vascular endothelial growth factor (VEGF) kit was used to determine the angiogenic effects with the obtained samples. As a result, in this study, it was observed that concentration and time-dependent boric acid decreased proliferation in MCF-7 breast cancer cell line. At the same time, according to the PARP results, it was found that the concentrations of boric acid significant differences compared to the control group. There was no significant difference between boric acid concentrations and Cis Platin groups. It is thought that both groups showed an increase compared to the control group, and boric acid leads the cell to necrosis like Cis Platinum. When VEGF results were examined to explain the relationship between boric acid and angiogenesis, significant differences were observed with both control group and Cis Platinum. Boric acid groups have been shown to be more effective than Cis Platin in the MCF-7 breast cancer cell line and to be an antiangiogenic agent. In this respect, further studies are needed to investigate how the effects of molecular anticancerogenic mechanisms of MCF-7 cells and boric acid on cell polarization are realized.

KEYWORDS: MCF-7, Breast Cancer, Boric Acid.

İNTRODUCTİON

Breast cancer is the most common disease that causes major health problems in women. It is in the first place among all cancer cases seen in women with a rate of 24,1% in our country.[1] Although the incidence and prognosis vary according to geographical regions, it is reported that the incidence of breast cancer increases by 1,5% every year.[2] It is reported in the literature that the most common type of breast cancer has the best survival because it has a 5-year survival rate of 75%.[3-4]

MCF-7 cells, a breast cancer cell line, were first isolated in 1970 from the pleural effusion of a 69-year-old woman of invasive ductal carcinoma.[5] This cell line was established by Soule et al. The name MCF-7 is used as the abbreviation of Michigian Cancer Foundation -7. MCF-7, a commonly studied epithelial cancer cell line derived from breast adenocarcinoma, has the characteristics of differentiated breast epithelium. Synthesize insulin-like proliferation factor binding proteins. Mutations in cyclin D, one of the cell cycle control points in the formation of breast cancer and many other cancers, are also present in MCF-7 cells. Expression of caspase 6, 7 and 9 and BCL-2 in the MCF-7 cell line is highly effective. In addition, expression and regulation of p53 and p21 genes are normal.[6]

Boron (B) is the fifth element of the periodic table and is the only non-metallic element in group 3A. Boron, which has a characteristic between metal and nonmetal, is not present as a free element in nature. It is present in combination with oxygen such as boric acid or borate salts. The atomic number is 5 and the atomic weight is

10.81. Boron is present in nature as a mixture of 10B (19.78%) and 11B (80.22%) isotopes. It is used in more than 250 varieties in industrial scale. These are mainly; glass, ceramics, cleaning and bleaching, flame retardants, agriculture, metallurgy, nuclear applications and health.[7] Boron (B); It is an important trace element that plays a role in cell membrane function, mineral and hormonal metabolism and enzyme reactions. Therefore Bor; It is also associated with osteoporosis, heart disease, stroke, diabetes, aging, and especially changes in the reproductive system.[8]

Boric acid, a boron derivative, is one of the most studied boron-containing chemicals. Boric acid has been shown to control the proliferation of certain types of cancer. A high dose of boric acid (12,5-50 mM) has been shown to slow cell replication and induce apoptosis in both melanoma cells and MDA231 breast cancer cells.[9-10] Thus, inhibition of cancer cells by boric acid includes a variety of cellular targets such as direct enzymatic inhibition, apoptosis, receptor binding and mRNA insertion. Barranco and Eckhert examined the anti-proliferative effects of boric acid in cell lines that cause prostate cancer, and consequently observed that high doses of boric acid have apoptotic effects.[11] Recently, 1 mM boric acid has been experimentally shown to inhibit the ZR-75-1 breast cancer cell line.[12] In this study, we aimed to investigate apoptotic, angiogenic and anticarcinogenic effects by studying different concentrations of boric acid on MCF-7 human breast cancer cell line based on this information.

MATERİALS AND METHODS

1.1. Cell Material

The human breast cancer MCF-7 cell line used in our research is from Celal Bayar University Faculty of Medicine, Histology and Embryology laboratory, it was obtained from Prof.Dr Mehmet İbrahim Tuğlu.

1.2. Cell Culture

Cells were seeded into sterile T25 and T75 flasks and cultured in a medium of 10% FCS, 10% RPMI 1640, 1% penicillin / streptomycin solution in a 5% CO 2 incubator at 37°C. A sufficient number of cells were seeded in 96-well plates with 5,000 cells per well and prepared for the experiments.

1.3. CCK-8 Prolification Analysis in MCF-7 Cells

We removed MCF-7 cells from the incubator that we passaged for cell proliferation analysis. RPMI containing 10% FBS and 1% penicillin / streptomycin was then added to the 96-well plate at 90 µl with an average of $5x10^5$ cells / ml. 5% CO 2 and 37°C were incubated in the incubator. After 24 hours of incubation, the wells were grouped and 10 µl of boric acid was added.

Table 1: Groups formed by addition of boric acid.

GROUPS	APPLİCATİONS
GROUP-1	%10 FBS ve %1 penisilin/streptomisin RPMI (medium)
GROUP-2	Cis Platin
GROUP-3	100 mM Boric Acid
GROUP-4	75 mM Boric Acid
GROUP-5	40 mM Boric Acid
GROUP-6	20 mM Boric Acid
GROUP-7	10 mM Boric Acid
GROUP-8	5 mM Boric Acid

For the proliferation assay, the wells were grouped as shown and the formed groups were incubated for 48 hours with three replicates. After completion of 48 hours, 10 µl of commercially available CCK-8 solution was added. Incubated for 2 hours. At the end of the incubation period, the plate was placed into the plate reader by gently shaking and measured at 450 nm.

1.4. PARP Analysis

After the cells covered 85-90% of the flask base, for the experiment was seeded in a 24-well plate with $1x10^5$ cells per well. After 24 hours, 10, 20, 40 mM boric acid doses were treated on the seeded to cells and allowed to incubate for 48 hours. After the incubation, the medium was removed and the cells were washed with cold PBS. After washing, PBS was removed. Trypsin was added to the cells to lift the cells. In order to eliminate the effect of trypsin, the cells transferred to the medium containing eppendorf were centrifuged at 15°C, 1600 rpm for 15 minutes. The supernatant was discarded after centrifugation. It was gently shaken by adding medium into eppendorf and made ready for PARP analysis. The single-use antibody loaded plate in the commercially available PARP kit was seeded and the kit procedure was performed. commercially obtained "Human PARP ELISA Kit" was used in the experiment. (Kit Lot No: 201904).

1.5. VEGF Analysis

After the cells covered 85-90% of the flask base, for the experiment was seeded in a 24-well plate with $1x10^5$ cells per well. After 24 hours, 10, 20, 40 mM boric acid doses were treated on the seeded to cells and allowed to incubate for 48 hours. After the incubation, the medium was removed and the cells were washed with cold PBS. After washing, PBS was removed. Trypsin was added to the cells to lift the cells. In order to eliminate the effect of trypsin, the cells transferred to the medium containing

eppendorf were centrifuged at 15°C, 1600 rpm for 15 minutes. The supernatant was discarded after centrifugation. It was gently shaken by adding medium into eppendorf and made ready for VEGF analysis. The single-use antibody loaded plate in the commercially available VEGF kit was seeded and the kit procedure was performed. commercially obtained "Human VEGF ELISA Kit" was used in the experiment. (Kit Lot No: 201812).

2. Statistical Analysis

In our study, ELISA device test results were analyzed using SPSS-18 statistical program. Results were given as mean (SE) standard error.(SE=SD/n).

After the homogeneity of the groups was tested, Tukey HSD and Duncan were used in one-way ANOVA test to find differences between groups. Differences were considered statistically significant at P<0.05.

RESULTS

2.1. CCK-8 Prolification Findings in MCF-7 Cells

As a result of literature review, the dose range of boric acid for MCF-7 human breast cancer cell line was determined as 5, 10, 20, 40, 75, 100. For the viability ratio the cells, the best results were obtained at the end of 24, 48 and 72 hours and the median lethal dose (LD50) was determined as 20 mM. Other experiments were based on this time and dose.

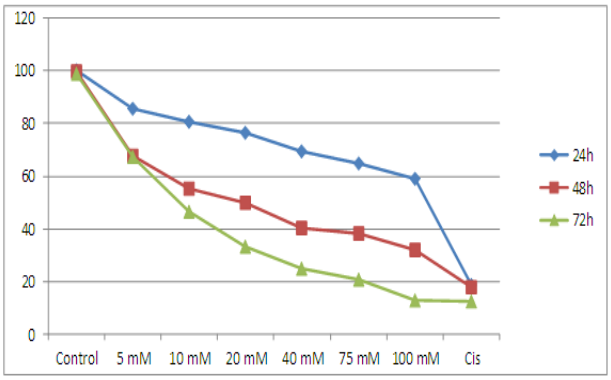

Figure 1: Viability - Concentration Graphic.

2.2. PARP and VEGF Analysis Findings

Boric acid concentrations of 10, 20, 40 mM were applied in the MCF-7 human breast cancer cell line and PARP and VEGF were analyzed by ELISA. The results are shown in the table.

Table 2: PARP and VEGF analysis results.

	PARP (ng\L)	VGEF (ng\L)
Control	0,348± 0,03[a]	0,96 ± 0,03[a]
Bor 10 mM	0,779± 0,07[b]	0,095 ± 0,01[b]
Bor 20 mM	0,768± 0,01[b]	0,098± 0,01[b]
Bor 40 mM	0,757± 0,05[b]	0,091 ± 0,05[b]
Cis Platin	0,736± 0,02[b]	0,82 ± 0,04[c]

**a,b,c: Means in the same column by the same letter are not significantly different according to the one way ANOVA-Duncan test (P<0.05).

DİSCUSSİON

Breast cancer is the most common type of cancer among women and ranks second among the causes of female death in the world. In order to find a solution to breast cancer, different studies are being conducted the treatment and the effects of different drugs and substances on this disease are intensively investigated. The aim of this study was to investigate the apoptotic, angiogenic and antioxidant effects of boric acid use in breast cancer cell line (MCF-7).

Chemotherapeutic agents damage neoplastic cells, while some normal cells are affected, causing various side effects. Boric acid has been shown to control the proliferation of certain types of cancer cells. Boric acid is an inhibitor of peptides, proteases, proteasomes, arginase, nitric oxide synthase and transpeptidases. Boric acid has a strong antioxidant effect, especially in reducing the side effects of drugs used in cancer treatment and reactive oxygen species (ROS) is important to reduce the level.[13]

Barranco and Echert determined proliferative inhibition by boric acid at 60 mM in the DU-145 prostate cancer cell line and observed that boric acid suppressed growth. Although this concentration is higher than pharmacological anti-cancer drugs, boron blood levels in this range have been reported in the human diet and pharmacological studies. Human blood levels have been reported to reflect dietary intake and range from 13 to 70 mM B.[11]

Scorei R. et al. Investigated the effects of boric acid and calcium fructobate on breast cancer cell line MDA-MB-231. The effects of different doses of boric acid and fructoborate (0.45-22.5 mM) on cell viability were investigated by 3- (4,5-dimethylthiazolyl-2) -2,5-diphenyltetrazolium bromide (MMT) method. CF and BA have been shown to induce a concentration-dependent cytotoxicity in MDA-MB-231 cells.[10]

Ceyhan et al DU-145 Human Prostate Cancer Cell Line high concentrations of boric acid oxidative stress, apoptotic pathways and morphological changes were investigated. The effects of boric acid (0–16,15 mM) applied at varying doses on cell viability after 24 hours were examined by MTT. It was found that boric acid-containing cells caused a statistically significant decrease in cell viability compared to control.[14] In our study, 5 mM, 10 mM, 20 mM, 40 mM and 100 mM boric acid were applied and a direct proportional decrease in cell proliferation was observed at 24, 48 and 72 hours in the MCF-7 breast cancer cell line. However, very high anti-proliferative effect was obtained from the 72th hour data. The best results were obtained at 48 hours, so other experiments were performed based on this time. The

effect of boric acid at different doses (0-100, 20 mM) applied to the cells was examined after 48 hours and we found that it caused a statistically significant decrease in cell viability compared to the control group.

PARPs are a family of 17 nucleoproteins characterized by a common catalytic site that transfers an ADP-ribose group on a specific acceptor protein using NAD+ as cofactor. Most PARP members are able to transfer only a mono-ADP ribose group to their target proteins, whereas PARP1, PARP2, PARP3, PARP5a, PARP5b characteristically add repeated ADP-ribose units, thus generating long poly (ADP-ribose) (PAR) chains.[15] This post-translational protein modification is named PARylation and allows PARPs involvement in different cellular activities. In this regard, PARP1 is the best characterized PARP.[16] PARP plays a role in DNA repair and transcriptional regulation and is currently considered to play a key role in cell survival and cell death. Furthermore, it is accepted as a master component of many transcription factors which are involved in tumor development.[17]

PARP, one of the first identified substrates of caspasehas vital role especially in apoptosis and necrosis. In the apoptosis process, caspase 7 and caspase 3 cleave PARP between Asp214 and Gly215 and break down p85 and p25. The PARP cleavage separates DBD from the catalytic domain and inactivates the enzymes. This eliminates PARP activation in response to DNA fragmentation during apoptosis and hinders the necessary ATP consumption in necrotic cell death and the futile efforts required for DNA repair. Thus, PARP cleavage helps the cell enter the apoptotic pathway and is recognized as the distinctive feature of apoptosis.[18,19]

In a study, the dose-dependent decrease of PARP protein in BPH-1 (Benign Prostate Hyperplasia Cell Line) cell lines treated with DPB (Disodium Pentaboratdecahydrate) for 48 hours showed that DNA did not enter the repair mechanism pathway. The opposite increase in PARP level indicates that the cells enter the apoptosis pathway.[20] PARP works according to the amount of damage in DNA. If DNA damage is very high, PARP leads the cell to necrosis with ATP / NAD consumption. If DNA damage is minimal, PARP, along with other DNA repair enzymes, can help the cell survive. If the caspase is activated, caspase, which plays a critical role in apoptosis from the caspase family and is the main responsible for the cleavage of PARP, goes into cell apoptosis by performing PARP cleavage.[21] In this study, PARP level was higher in MCF-7 breast cancer cell line compared to control group and there was a significant difference between them. No significant difference was observed between the cisplatin group. As a result of the experiment PARP values were higher in cisplatin group and boric acid concentration groups. This is due to the overactivation of PARP in cisplatin as a result of increasing NAD and ATP consumption, the cell is thought to lead to necrosis. We think likewise, boric acid also raises the parp level leads to cell necrosis.

Vascular endothelial growth factor (VEGF), known as vascular permeability factor (VPF), is defined as endothelial cell-specific mitogen. VEGF is produced by many cell types, including tumor cells, platelets, macrophages, keratinocytes and kidney mesangial cells. The activities of VEGF are not limited to the vascular system; VEGF plays a role in physiological functions such as hematopoiesis, bone formation, wound healing and development.[22] According to the article published in 2009, high serum VEGF levels increased in endometrial and breast cancers. However, it can be elevated due to benign causes such as fibroadenoma or endometrial hyperplasia. Furthermore, these elevated serum VEGF levels were increased in tumor cells. It was found that VEGFR-2 / KDR activity was closely related and involved in tumor progression.[23]

Pedro et al. in a study published in 2012, preoperative and postoperative serum VEGF and VEGF-C levels were compared in gastric cancer patients and a significant decrease was observed postoperatively. Based on this finding, it is thought that the follow-up of serum VEGF level may be useful in showing progression, high preoperative serum VEGF levels may be associated with poor prognosis and neoadjuvant therapy may be beneficial in these patients.[24] In a study performed in patients with colon cancer, serum VEGF-C and tissue VEGF-C expression was shown to be associated with increased lymph node metastasis and poor prognosis.[25]

In 2011, Ito et al. Published two separate breast cancer tissue cultures with high and low metastasis potential in the breast tissue of two mice of the same species and examined LVD and VEGF-C levels. The mouse with high malignancy potential showed more LVD and increased VEGF-C levels. Concomitant increased axillary lymph node metastasis.[26] In this study, VEGF values in MCF-7 cancer cell line showed significant differences as high concentrations reached. We think boric acid has dose-dependent antiproliferative effects and prevents angiogenesis.

We think that chemotreapic studies against cancer are carried out on many active substances, boron's is used in many different fields in cancer treatment and molecular effects of boron supplementation should be elucidated with further studies.

REFERENCES

1. T.C. Sağlık Bakanlığı Kanserle Savaş Politikası ve Kanser verileri, (1995–1999), Kanser Savaş Dairesi Başkanlığı Bakanlık Yayın No, 2002; 168.
2. Yılmaz, MR. Meme kanserinin epidemiyolojisi ve etiyolojisi. Esin Emin Üstün (ed). Meme kanseri. Ayın Kitabı, Ege Üniversitesi Tıp Fakültesi Dekanlığı, Yayın Bürosu, İzmir, 2002; 25-40.

3. Henson, HK. Meme Kanseri ve Cinsellik. Sexuality and Disability, 2002; 20(4): 261-275. Çeviri: D. Aygin, Androloji Bülteni, say, 2004; 19: 366- 7.
4. ThorsCL, Broeckel JA, Jacobsen PB. Sexual Functioning in Breast Cancer Survivors, Cancer Control, 2001; 8(5): 442-448.
5. Soule, HD, Vazguez J, Long A, Albert S, & Brennan M. A human cell line from a pleural effusion derived from a breast carcinoma. J NatlCancerInst, 1973; 51(5): 1409-1416.
6. Chodosh, LA. Ekspression of BRCA1 and BRCA2 in normal andneoplasticcells. Journalof Mammary Gland Biology and Neoplsia, 1998; 3: 389-402.
7. Martin AM, Weber BL. Geneticand hormonal risk factors in breast cancer. Journal of the National Cancer Institut, 2000; 92: 1126-1135.
8. Martin SP, Pike MC, Ross RK, Jones PA, Henderson BE. Increased cell division as a cause of human cancer. Cancer Research, 1990; 50: 7415-7421.
9. Acerbo, A.S. & Miller, L. Assessment of the chemical changes induced in human melanoma cells by boric acid treatment usingin fraredim aging. Analyst, 2009; 134: 1669-1674.
10. Scorei, R.;Ciubar, R.; Ciofrangeanu, C.M.; Mitran, V.; Cimpean, A. &Iordachescu, D. Comparative effects of boric acid and calcium fructoborate on breast cancer cells. Biological Trace Element Research, 2008; 122: 197-205.
11. Barranco, W. T., & Eckhert, C. D. *Boric acid inhibits human prostate cancer cell proliferation. Cancer Letters*, 2004; *216(1): 21–29.* doi:10.1016/j.canlet.2004.06.001.
12. Bradke, T.; Hall, C.; Stephen, W.; Carper, S.W.; Plopper, G.E. Phenylboronic acid selectively inhibits human prostate and breast cancer cell migration and decreases viability. Cell Adh. Migr, 2008; 2: 153-160.
13. Ward N.L. The determination of boron in biological materials by neutron irradiation and prompt gamma-ray spectrometry, J. Radioanal. Nucl. Chem, 1987; 110: 633–639.
14. Sevimli M, Investigating the effects of boric acid on colon cancer cell lines in in vitro conditions (Doctoral thesis, Suleyman Demirel University, 2018; 157.
15. Barkauskaite E, Jankevicius G, Ahel I. Structures and mechanisms of enzymes employed in the synthesis and degradation of PARP-dependent protein ADP-ribosylation. Mol Cell, 2015; 58: 935–46.
16. Weaver AN, Yang ES. Beyond DNA repair: additional functions of PARP-1 in cancer. Front Oncol, 2013; 3: 290.
17. Peralta-Leal A, Rodríguez-Vargas JM, Aguilar-Quesada R, Rodríguez MI, Linares JL, de Almodóvar MR, Oliver FJ. PARP inhibitors: New partners in the therapy of cancer and inflammatory diseases. Free Radic Biol Med, 2009; 47(1): 13-26.
18. Elisa Cleaved PARP Kit, Katalog No: KHO0741, Invitrogen Corporation, Kalifornia, A.B.D. (www.invitrogen.com), 2011.
19. Chiarugi A., Moskowitz M. A. PARP-1-a perpetrator of apoptotic cell death, Science, 2002; 297(5579): 200-201.
20. Folkman J., Angiogenesis, Annu. Rev. Med, 2006; 57: 1-18.
21. Boulares A. H., Yakovlev A. G., Ivanova V., Stoica B. A., Wang G., Iyer S., Smulson M., Role of Poly(ADP-ribose) Polymerase (PARP) Cleavage in Apoptosis, J. Biol. Chem., 1999; 274(33): 22932–22940.
22. Angela M. Duffy, David J. Bouchier-Hayes, and Judith H. Harmey. Vascular Endothelial Growth Factor (VEGF) and Its Role in Non-Endothelial Cells: Autocrine Signalling by VEGF, 2011; 79.
23. Michael I. Koukourakis, Vassilios Limberis, Ioannis Tentes, Emmanuel Kontomanolis, Alexandros Kortsaris, Efthimios Sivridis, Alexandra Giatromanolaki Cytokine 53 Serum VEGF levels and tissue activation of VEGFR2/KDR receptors in patients with breast and gynecologic cancer, 2011; 370–375.
24. Pedro Villarejo-Campos, David Padilla Valverde, Rau´l Martin Martin, Pablo Mene´ndez-Sa´nchez, Teo´filo Cubo- 44 Cintas, Jose Antonio Bondia-Navarro, Jesu´ s Martı´n Ferna´ndez; Clin Transl Oncol, Serum VEGF and VEGF-C values before surgery and after postoperative treatment in gastric cancer, 2013; 15(4): 265-70.
25. Tian-Bao Wang, Zhong-Gang Chen, Xiu-Qing Wei, Bo Weiss and Wen-Guang Dong; ANZ J Surg 81 Serum vascular endothelial growth factor-C and lymphoangiogenesis are associated with the lymph node metastasis and prognosis of patients with colorectal cancer, 2011; 694–699.
26. Yuko Ito, Masa-Aki Shibata, Nabil Eid, Junji Morimoto, and Yoshinori Otsuki; International Journal of Breast Cancer Volume. Lymphangiogenesis and Axillary Lymph Node Metastases Correlated with VEGF-C Expression in Two İmmuno competent Mouse Mammary Carcinoma Models., Article ID 867152, 2011; 10.