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Cytotoxic Effect of Boron Application on Glioblastoma Cells

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ABSTRACT

AIM: To investigate the cytotoxic effects of boron application at different doses on U-87 MG glioblastoma cells.

MATERIAL and METHODS: The T98G (ATCC® CRL-1690™) glioblastoma cell strain used in the study was acquired from the American Type Culture Collection (ATCC) (Manassas, USA). Boric acid solution was prepared by mechanical mixing in the medium. Afterwards, 2.5 mM, 25 mM and 50 mM boron were each added to U87-MG glioblastoma cells and incubated for 48 hours. The cytotoxic effects on the cells was determined using the MTT (Methylthiazole diphenyl tetrazolium) test 48 hours after boron application.

RESULTS: IC50 value was detected as 17 mM in the 48-hour boric acid application on U-87 MG glioblastoma cells.

CONCLUSION: Boron treatment might be an effective approach for glioblastoma.

KEYWORDS: Boron treatment, Glioblastoma, Cytotoxic effects

ABBREVIATIONS: ATCC: American type culture collection, **MTT:** Methylthiazole diphenyl tetrazolium, **GBM:** Glioblastoma, **BNCT:** Boron neutron capture therapy, **EMEM:** Eagle's minimum essential medium, **FBS:** Fetal bovine serum, **FDA:** Food and Drug Administration

INTRODUCTION

As the most aggressive form of glioma, glioblastoma (GBM) is the most common primary brain tumour in adults (31). The World Health Organization classifies it as a stage IV glioma and its prevalence is approximately 3/100.000 individuals (1).

It remains a mortal disease with an extremely low prognosis despite the availability of different modern GBM treatments (27). Regarding standard surgical treatment application, radiotherapy and chemotherapy have been applied. With a highly invasive character, glioblastoma cells do not only escape surgery and focal treatments, but also resist the available radio- and chemotherapeutic approaches (13). A survival rate of approximately 15 months after diagnosis (32), and being a disease with a poor prognosis make glioblastoma an important public health problem. Thus, it is important to develop new treatment strategies that prevent the invasiveness of glioblastoma in order to treat this mortal disease.

New glioblastoma treatments are being developed and tested. Tumour vaccine treatment in GBM aims to promote the immune systems of the patients to produce immune cells by transferring tumor-related antigens. In this regard, Dendritic cell and heat shock protein vaccine treatment studies are ongoing (8,10).

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Tumor cells are infected and destroyed by oncolytic viruses. Clinical studies have shown that the use of recombinant adenovirus DNX-2401, poliomyelitis-rhinovirus chimera and parvovirus H-1 lengthens the survival in GBM patients (30).

Boron neutron capture therapy (BNCT) kills tumour cells selectively, depending on the preloaded boron neutron capture reaction during low energy thermal neutron irradiation of the tumour cells (20).

In addition, boron element, which can be taken with the daily diet and can easily be applied with the available treatments with proven anticarcinogenic effects can be used for glioblastoma treatment. Boron compounds taken orally are quickly turned into boric acid in the gastrointestinal system and distributed to the tissues through the blood. Boron atoms in biological systems interact with proteins through strong hydrogen and weak covalent bonds providing biological effects (12). Natural boron compounds with antibacterial, antiviral and anticancer activities are used for treatment in pharmaceutic formulations (5,28). Due to the high proliferative rates and metabolisms, cancer cells need a lot of energy. Boron damages cancer cells by closing the primary energy gain paths in prostate, cervical, lung and esophageal cancers (6). Thus boron compounds deserve to be examined within the scope of neurological and oncological diseases in future studies.

U-87 MG cell strain covers epithelial cells derived from human malignant gliomata and U87MG is commonly used in human glioma research. U87-MG cell strain preferred by many research groups is suggested as a classical cell strain for glioblastoma (4). The aim of this study was to investigate the cytotoxic effects of boron application on U-87 MG glioblastoma cells.

MATERIAL and METHODS

U87-MG Glioblastoma Cell Culture

T98G (ATCC® CRL-1690™) glioblastoma cell strain used in the study was acquired from the American Type Culture Collection (ATCC) (Manassas, USA). Before starting the cell culture study, EMEM (Eagle's Minimum Essential Medium containing ATCC, USA) medium + 10% Fetal Bovine Serum (FBS; ATCC, USA) + penicillin/streptomycin (100 µg/ml; Gibco, US) was prepared in a ultraviolet cabin under sterile conditions.

Boron Application in U87-MG Glioblastoma Cells

Boric acid solution was prepared through mechanical mixing in the medium. Afterwards, 2.5 mM, 25 mM and 50 mM boron were each added to the U87-MG glioblastoma cells and incubated for 48 hours. Only culture medium was added to the control cells.

MTT Test Application in U87-MG Glioblastoma Cells

The ratio of viable cells in the cell strain was colorimetrically detected using the MTT [3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl Tetrazolium Bromide] method. The test was performed according to the principle of yellow MTT stain emerging by disruption of the tetrazolium ring turning into dark blue-purple formazan product. Cells were incubated in 35 mm x 10 mm sterile petri dishes at 37°C and 5% CO_2. Different doses of boron were applied on the cells. We placed 2 ml of medium containing 0.5 µg/µl MTT and 0.5% FBS in each dish and incubated for four hours. After the four hours, MTT medium in the petri dishes was aspirated and 200 µl 3% SDS was added into each petri dish and mixed. Then 1 ml 40 mM Hcl/isopropanol was added and mixed. The cells were homogenised with a pipette and HCl/isopropanol was used for dilution. The stain volume acquired at the end of analysis was measured at 570 nm wavelength absorbance using SmartSpec Plus spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the viable cell ratio was determined (34). GraphPad Prism 5.0 program (GraphPad Software, Inc., La Jolla, CA, USA) was used for data analysis and a graphic was formed. To calculate the IC_{50} value, data were normalized by nonlinear regression analysis using the GraphPad Prism 5.0 program.

Cell Viability Analysis

Cell viability ratios were calculated in comparison with the untreated control cells to determine the effect of boron application. Viability of the untreated cells was regarded as 100% and the viability percentages of the cells were calculated as follows:

% viability ratio: (Treated cell/ untreated cell) X 100

RESULTS

The cells were cultured in a standard medium for 24 hours before boron application on the U87-MG Glioblastoma cells. Later, 2.5 mM, 25 mM and 50 mM boron was added to each cell culture medium and incubated for 48 hours. Only culture medium was added to the control cells. MTT test was performed on the cells after boron application. Using the GraphPad Prism 5.0 program, statistical analysis was conducted from the data acquired through the MTT test. Based on the statistical analysis following 2.5 mM, 25 mM and 50 mM boron application on glioblastoma cells for 48 hours, IC_{50} value of boron in the 48^{th} hour was calculated as 17 mM (Figure 1). This result shows that boron has a cytotoxic effect on U87-MG glioblastoma cells.

To analyse the percentage survival ratios of U87-MG glioblastoma cells due to the application of boron at different doses, 2.5 mM, 25 mM and 50 mM boron were applied for 48 hours on the multiplied cells until it reached the logarithmic phase in 35 x 10 mm petri dishes. Then, the MTT cytotoxicity test was performed (Figure 2). Compared to the controls, the percentage cell viability was calculated to be 90% as the result of the 2.5 mM boron application, 46% for the 25 mM boron application and 23% for the 50 mM boron application. In addition, the 50 mM boron was found to have a fatal effect on glioblastoma cells. It was found that 25 mM but not 2.5 mM boron application had a multiplication-preventing effect on U87-MG cells (Figure 2). High dose boron application was observed to have a cytotoxic effect on glioblastoma cells.

DISCUSSION

Surgery, adjuvant radiotherapy and chemotherapy are among the current treatment options for glioblastoma patients. Based

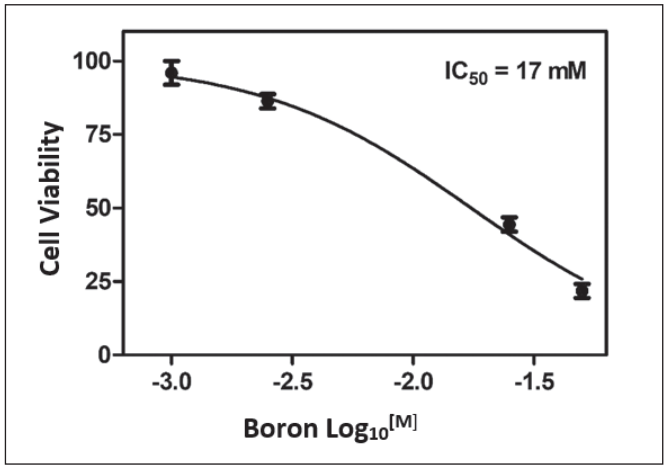

Figure 1: Boron IC_{50} value in U87-MG glioblastoma cells and the % survival graphic. IC_{50} value of the cells treated with different concentrations of boron for 48 hours was detected with the MTT test.

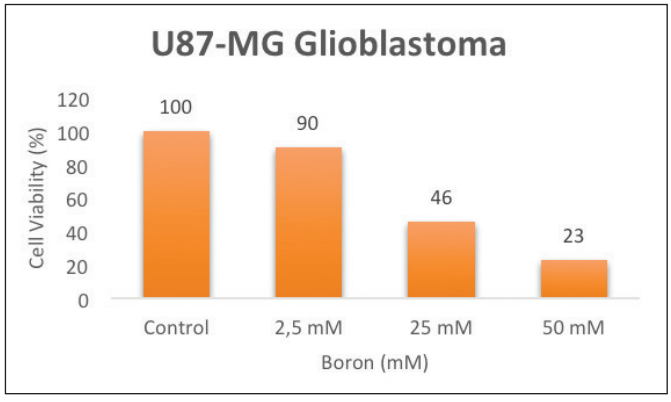

Figure 2: Cytotoxic effect of boron application at different doses on U87-MG cells. MTT test was performed after treating the U87-MG cells with 2.5 mM, 25 mM and 50 mM boron for 48 hours.

on previous studies, the median survival period is extended by approximately two months when radiotherapy is applied in addition to chemotherapy following surgical treatment (16,17). It is suggested that more research is required to determine its therapeutic strategies in alternative *in vitro* and *in vivo* models in addition to the current treatments to extend survival.

Boron atoms present an antibacterial, antiviral and anticancer character by interacting with proteins through hydrogen and covalent bonds in living systems (6). Due to their constantly dividing nature, cancer cells have high metabolic activities and need more energy compared to healthy cells. Boron compounds cause less energy production by affecting the biochemical cycles that produce energy in cancer cells, thereby negatively affecting the proliferation of cancer cells (22).

Being common in nature, boron and its compounds can also be taken through the diet. Previous studies have shown that boron may affect the brain functions and cognitive performance (26). The brain is among the parts of the body where boron compounds are most commonly found after being taken into the body. In the experiment by Ku et al., it was reported that boron I reached a constant level in the brain and hypothalamus in addition to some other parts of the body, after a boric acid diet for seven days (23). In another study, boric acid level in the gray substance of the brains of laboratory animals sacrificed after boric acid diet was found to be higher than those in the white substance (25). It was observed that boric acid treatment significantly decreased tissue damage, inflammation and oedema in an ischemia/reperfusion model of the spinal cord (21).

Boron compounds have started to be used in studies on different cancer types in recent years. The cytotoxic effects of boron derivative compounds such as boric acid, phenylboronic acid, dihydroxy boron and sodium tetraborate were detected on B16F10 murine melanoma, HL-60 and U-937 human leukemia cells (11,14). In their study, Mahabir et al. stated that high boron intake had a preserving effect against lung cancer (24). In a study investigating the effect of boron application on H1299 and COR-L23p lung cancer at different doses, Al-Ali and Gonzalez-Sarmiento reported that 5 mM boric acid concentration did not create a significant effect on cell growth, but 25 mM boric acid concentration inhibited cells (2). In a study investigating the 24 hour effect of boric acid on DU-145 human prostate cancer cell strain, Hacioglu et al. showed that the cell viability ratio was 61% in 6.25 mM boric acid concentration and 45.8% in 12.5 mM boric acid concentration (18). In a study investigating the cytotoxic effects of boron trioxide on L929 fibroblast cells and DLD-1 colorectal adenocarcinoma cells, Albuz et al. found that the cytotoxic activity was 55.78% for DLD-1 and 81.64% for L929, when 10 µg/mL concentration was used (3). This condition shows that the cancer cells are more resistant than normal cells.

In their study investigating the effects of highly water-soluble nano-structured boron nitride compounds which can be used in BNCT on HeLa (cervical cancer) and MCF-7 (human breast adenocarcinoma) cells, Singh et al. found cytotoxicity ratios of 60% and 45%, respectively, for 2 mg/mL drug dose (29). Wittig et al. reported that boron use for GBM was promising for BNCT research in the study investigating the ^{10}B-accumulation in human glioblastoma multiforme (U87MG) cells (33). Based on the studies in literature, application of high dose boron/boron compounds in different cancer types demonstrates a cytotoxic effect in cells, similar to the finding of our study. We found that the survival ratio was 25% for 50 mM, 46% for 25 mM and 90% for 2.5 mM boron application on U87-MG cells.

Boric acid is the main product of boron following hydrolysis after oral intake. Bortezomib [N-2,3-pyrazine)carbonyl-L-phenylalanine-L-leucine-boronic acid] is the first Food and Drug Administration (FDA) approved antineoplastic which is made of boron and is effective through proteasome inhibition. It is used especially in multiple myeloma and non-hodgkin lymphoma treatment. Peak plasma concentration of Bortezomib is 25-50nM (6,35).

In their study, Bittencourt et al. proved that Bortezomib had an antiangiogenic effect on U87 cells at 20 nM (7). In our study, 25 nM and 50 nM were also found to be effective.

In previous animal studies, it was shown that boric acid inhibited Histon deacetylase functioning in regulating gene expressions. Histon deacetylase inhibition prevents cancer formation by inhibiting cell growth (15). It was considered that this characteristic could be used for cancer treatment. As a weak Lewis acid, boric acid also resembles a carbon atom chemically (9). Being able to easily enter the cells with its small atom diameter, boric acid functions as a drug delivery vehicle in anti-cancer treatment. Boron capture therapy is its most famous example. In addition to these characteristics, having a low-toxicity character, excretion in a short period through urine without being metabolized and easy acquisition gives it an advantage over other medical therapies in cancer treatment (19).

In our study, while 50 mM boron application had a lethal effect on glioblastoma cells and 25 mM boron application had a multiplication-preventive effect on U87-MG cells, we found that 2.5 mM boron application did not show a multiplication-preventive effect on glioblastoma cells. High dose boron application was observed to have a cytotoxic effect on glioblastoma cells. Hacioglu et al. found IC_{50} rate as 10.77 mM in boric acid application with different concentrations of 12.5, 6.5, 3.13, 1.56, 0.78, 0.39 and 0.19 mM for 24 hours on DU-145 cells (18). Based on the statistical analysis, after 2.5 mM, 25 mM and 50 mM boron application on glioblastoma cells for 48 hours, IC_{50} value of boron in the 48th hour was calculated as 17 mM. This result shows that boron has a cytotoxic effect on U87-MG glioblastoma cells.

CONCLUSION

GBM is a disease with a poor prognosis due to the limitations and lack of significant improvements in its treatment. Considering its complexity and treatment difficulties, new therapeutic approaches are needed to improve the results for the patients. We investigated the cytotoxic role of boron application on GBM treatment in our study and found that high-dose boron application had a fatal effect on GBM cells and boron application at non-toxic doses did not inhibit cell proliferation. As a result of our study, it was shown that high-dose boron application is a promising approach for GBM treatment. We think that our findings provide an important background for future studies. More studies are needed to determine the efficiency of boron application in GBM treatment.

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