Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2023;12(1):26-31

Potential cytotoxic effects of borax alone and in combination with irinotecan on YKG1 glioblastoma cell-line

Yasemin Yuksel[1], Sefa Celik[2], Esra Aslan[3], Murat Tosun[3], Mukremin Uysal[4], Mehmet Bilgehan Pektas[5]

[1]Ankara City Hospital, Center of Assisted Reproduction, Obstetrics and Gynecology Clinics, Ankara, Türkiye
[2]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, Afyonkarahisar, Türkiye
[3]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, Afyonkarahisar, Türkiye
[4]Medstar Hospital, Department of Medical Oncology, Antalya, Turkey
[5]Afyonkarahisar Health Sciences University, Faculty of Medicine,Department of Medical Pharmacology, Afyonkarahisar, Türkiye

Received 13 December 2022; Accepted 08 January 2023
Available online 19.01.2023 with doi: 10.5455/medscience.2022.11.253

Abstract

Glioblastoma Multiforme (GBM), which is a common and primary brain tumor in adults, is an important cause of death worldwide as an aggressive and treatment-resistant cancer tumor. In this cell culture study, the apoptotic and anti-proliferative effects of borax and irinotecan at different doses, alone or in combination, were investigated in the YKG1 cell line. Cytotoxic activities were analyzed by MTT method and TUNEL staining after 24th and 48th hours of incubation with borax administered at doses of 1mg and 3mg per ml; irinotecan 50mM and 100mM. Both irinotecan and borax have been shown to induce apoptosis when used alone, and thus cause anti-proliferation. It was determined that these effects were potentiated by the combined application of the agents. In addition, it was determined that this effect in combined applications was more pronounced after 48 hours and at higher doses. In light of the data obtained, the combination of irinotecan with borax to increase the cytotoxic effect of irinotecan, which is used in many different cancer types, can be tried in further prospective studies.

Keywords: YKG1, irinotecan, borax, MTT, tunel

Introduction

Brain tumors are the third most common tumor in children and the eighth most common in adults. According to its pathogenesis, the most often brain tumor in adults is Glioblastoma Multiforme (GBM), which constitutes 35-45% of primary brain tumors. It is highly aggressive and patients with malignant glioma show unsatisfactory clinical prognosis and low survival rate [1,2]. Despite technological advances, the average survival time after diagnosis is around 12-15 months due to high invasiveness and heterogeneity. GBM treatment is very difficult because it is resistant to both radiation therapy and chemotherapy, and there is no radical solution to prevent cell growth that causes tumorigenesis. Therefore, current treatment protocols for tumors due to GBM include surgery if possible; including radiation therapy and chemotherapy [3,4]. However, regardless of applicability, combined chemotherapy is highly recommended [6,7]. One of the agents used for this purpose, irinotecan, provides Topoisomerase-I inhibition, which has a role in DNA transcription. Irinotecan diffuses across the blood-brain barrier and thus provides cytotoxic activity against brain tumors such

CITATION
Yuksel Y, Celik S, Aslan E, et al. Potential cytotoxic effects of borax alone and in combination with irinotecan on YKG1 glioblastoma cell-line. Med Science. 2023;12(1):26-31

Corresponding Author: Mehmet Bilgehan Pektas, Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Pharmacology, Afyonkarahisar, Türkiye
Email: mbpektas@gmail.com

DOI: 10.5455/medscience.2022.11.253

as GBM. In addition, irinotecan is known to be effective against multi-drug-resistant glioblastoma cells. However, increased efficacy of irinotecan has been demonstrated when used with other chemotherapeutic agents [8–10]. Combination chemotherapy targeting the disease from different mechanisms has recently been investigated to develop more effective treatment protocols.

Borax whose chemical formula is sodium tetraborate decahydrate, is a common form of boron in nature and obtained from different natural sources [11]. It has beneficial effects in medicine such as antiseptic, antifungal, and antiviral and also anti-osteoporotic, anti-inflammatory, hypoglycemic, and anti-coagulant [12,13]. The current literature indicates that borax has antioxidant properties with its free radical scavenging activity, inhibits proliferation in tumor cells, and therefore may exhibit anticancer effects by inducing apoptosis [14–18]. The mechanism underlying the anti-proliferative activity of borax has not been elucidated. However, there are not many studies investigating the biological activity of borax, especially in terms of its cytotoxicity in cancer cells. For these reasons, borax can be evaluated as an agent that can potentiate the effect of irinotecan. In this study, we investigated the possible effects of borax for enhancing the tumoricidal effects of irinotecan at different concentrations and combinations in human glioblastoma cell lines for 24 and 48 hours.

Material and Methods

Reagents

RPMI1640 medium (R8758), FBS (F9665), Penicilin-Streptomycin (P4333), L-glutamine (G7513), and Thiazolyl Blue Tetrazolium Bromide (M2128) were purchased from Sigma (Steinheim, Germany). Irinotecan (14180) was purchased from Cayman Chemical Company (USA). Borax (S9640) was obtained from Sigma (Steinheim, Germany). A Tunel assay kit was obtained from Millipore (S7101, MA, USA).

Cell Culture

YKG1 human glioblastoma cell line (RIKEN cell bank, Tsukuba, Japan) was used for the experiment. Cells were placed into 75 cm^2 culture flasks and produced in RPMI-1640 containing L-glutamine suffixed streptomycin (100 µg/ml), penicillin (100 U/ml), 10% fetal bovine serum (FBS), and 10 mM HEPES at 37°C with 5% CO2 in a humidified cell culture incubator. The medium was replaced every 3 days. Glioblastoma cells were incubated with determined concentrations and combinations of drugs (borax, irinotecan, borax + irinotecan) for the indicated time periods (24 and 48 hours). Three separate wells were used for each dose administration. Irinotecan doses were established according to the previous study [19], and borax' doses were established according to our preliminary study [20].

Experimental design

The study was designed as follows:

Group 1: Control group (no treatment, only 0.1% DMSO was used as a solvent). *Group 2:* Borax administered groups (at concentrations of 1mg/ml, 3mg/ml). *Group 3:* Irinotecan administered groups (at concentrations of 50mM, 100mM). *Group 4:* Combination groups of borax and irinotecan, respectively (1mg/ml + 50mM; 3mg/ml + 50mM; 1 mg/ml + 100 mM; 3mg/ml + 100mM.

Cell viability assay

MTT(3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay was used for detecting cell viability. MTT assay is a colorimetric survival assay based upon enzymatic degradation of the MTT formazan molecule in living cells. The purpose is to determine the percentage of surviving cells compared to the control after application. For this purpose, YKG1 cells were seeded into 96-well plates containing 100 µl cell suspensions at a concentration of 2x104 cells/ml for performing MTT. YKG1 cells were exposed to determined concentrations of borax, irinotecan, and borax + irinotecan combinations before the cytotoxicity determination and incubated for 24 and 48 hours at 37°C. DMSO was used for the control group. 20 µl MTT (5mg/ml) was added to the wells and plates were incubated again for 24 h at 37°C after 24 and 48 hours' of incubation. Then MTT dye was removed and 150 µl/well of DMSO was joined to each well and incubated for 10 min in the dark at room temperature. Finally, the plates were analyzed using a microplate spectrophotometer (Epoch, Biotek Inst., Winooski, USA) at a wavelength of 5740 nm. All experiments were performed three times, mean values were calculated and the percentage of cell viability was calculated for each group. Finally, control cell viability, not treated with components, was considered to be 100% and the viability of experimental cells was calculated as follows;

Cell viability (%) = OD treatment/OD Control x 100.

Detection of apoptosis by Tunel method

Tumor cell apoptosis was examined by the Terminal Deoxynucleotidyl Transferase dUTP Nick-end Labeling (Tunel) method. The apoptotic activity of GBM cells was defined as the apoptotic index (APOI). APOI was calculated by proportioning the non-apoptotic cells to the apoptotic cells. Tunel assay was performed with in situ apoptosis detection kit Apoptag® (Millipore, S7101) according to the manufacturer's protocol. Briefly, cells were seeded into 24-well plates and incubated for 24 and 48 hours after drug administration. Cells were taken to tubes at a density of 1x106/ml per well and centrifuged for 5 min and treated with PBS and fixed with 4% formaldehyde, and cells were centrifuged for 5 min again. Subsequently, the cells were re-suspended in 80% alcohol and taken to Poly-L-sine coated slides and permeabilized with Proteinase-K (100 µg/ml) and washed with TBS. Inactivation of endogenous peroxidase was provided with 3% H_2O_2 for 5 min. After washing with TBS, cells were incubated with Equilibration buffer at room temperature and TdT enzyme was added to the cells in a humidified chamber at 37°C for 60 min. Then stop wash buffer for 10 min and subsequently anti-digoxigenin conjugate for 30 min. were applied. Then slides

DOI: 10.5455/medscience.2022.11.253

were washed with TBS and DAB was used to label the apoptotic cells. Then the slides were rinsed in water and counterstained with methyl green and after dehydrating, cleaned with xylene and mounted with Entellan (Sigma). Finally, slides were evaluated under a light microscope using an image analysis program (NIS Elements, Nikon, Japan). Apoptotic and non-apoptotic cells were counted under x40 magnification in six different areas and APOI was calculated by proportioning the non-apoptotic cells to apoptotic cells.

Statistical analyses

MTT results were subjected to one-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS 21.0, SPSS Inc., Chicago, IL, USA) software. Differences among the groups were obtained using Tukey's test option. All data were expressed as mean ± standard error mean (SEM) in each group. Statistical significance was accepted as $p<0.05$.

Results

Cytotoxicity results

The results of the viability test performed in the 24th and 48th hours are shown in Table 1 and Figure 1. According to these results, the application of alone irinotecan has had an effective cytotoxic effect on glioblastoma cells. While this cytotoxic effect did not show a significant change dose-dependently in the 24th hour, it gradually increased dose-dependently in the 48th hour. According to MTT results, an evident cytotoxic effect was observed after the application of borax alone both in the 24th and 48th hours on glioblastoma cells. In the borax alone-treated groups, an increase in cytotoxicity on YKG1 cells was observed parallel to the increasing doses both in the 24th and 48th hours. Cytotoxic effect on YKG1 cells caused by borax alone was more prominent than irinotecan alone treatment. In the combination groups formed by different doses of irinotecan and borax, it was observed that the combined use of irinotecan and borax potentiated each other's cytotoxic effects to some extent in comparison to applications of irinotecan and borax alone. Cytotoxic effect was less than at lower dose combinations, it was observed to be higher at higher dose combinations. In MTT results after 24 hours, the most cytotoxic effect were observed in the combination of borax (1mg/ml) + irinotecan (50 mM) administered group. Looking at MTT results, more pronounced cytotoxicity on the YKG1 cell line was observed at the 48th hour compared to the MTT results at the 24th hour. The most cytotoxic effects were detected in combinations of borax (1mg/ml) + irinotecan (50mM), borax (1mg/ml) + irinotecan (100 mM) and borax (3mg/ml) + irinotecan (10mM). Evaluating the effectiveness of the combined use of irinotecan and borax in the 24th and 48th hours, it has been proved by the ratio of dead cells detected in the 48th hour being significantly higher than in the 24th hour.

Tunel assay results

APOI was calculated by dividing the number of non-apoptotic cells by the number of apoptotic cells. AI results of the groups are shown in Figure 2 and images of the Tunel assay are shown in Figure 3. According to these results, the application of irinotecan alone caused a dose-dependent increase in apoptotic cell death in the glioblastoma cells both in the 24th and 48th hours. The application of borax alone was observed to enhance apoptotic cell death dose-dependently both in the 24th and 48th hours at similar values to the administration of irinotecan alone. Apoptotic cell death was observed considerably higher in the groups of irinotecan and borax administered alone at the 48th hour compared to the 24th hour and also apoptotic cell death was higher in combination groups of irinotecan and borax compared to irinotecan and borax were administered alone. As the weakest effect inducing apoptosis in combined use was observed at lower combination doses, the strongest effect was detected when borax and irinotecan were administered at the doses of borax (1mg/ml) + irinotecan (50mM), and borax (1 mg/ml) + irinotecan (100 mM) and borax (3mg/ml) + irinotecan (100 mM) both in the 24th and 48th hours.

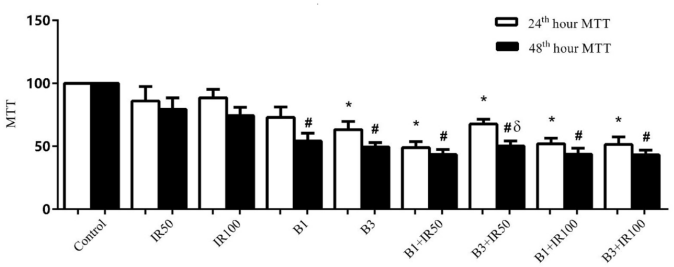

Figure 1. Cytotoxic activities of Borax (B) and Irinotecan (IR) with by MTT assays on glioblastoma YKG1 cells at 24th and 48th hours. Values are expressed as mean ± SEM. Control group (no treatment, only 0.1% DMSO was used as a solvent). Irinotecan administered groups IR50 (50mM) and IR100 (100mM); Borax administered groups B1 and B3 (at concentrations of 1 mg/ml and 3mg/ml, respectively). δ shows a significant difference from the 24th hours of each group. ($p<0.05$); *shows significantly difference from the 24th hours of Control ($p<0.05$); # shows significantly difference from the 48th hours of Control ($p<0.05$)

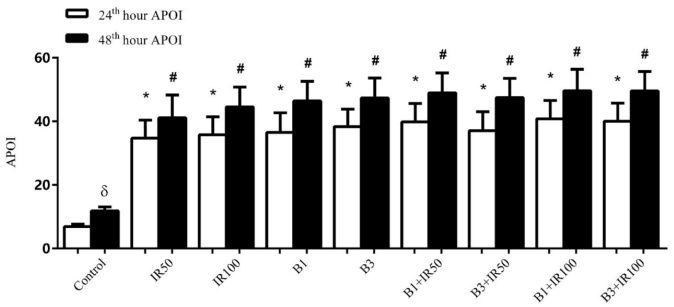

Figure 2. Apoptotic index (APOI) of Borax (B) and Irinotecan (IR) with by MTT assays on glioblastoma YKG1 cells after Tunel assay at 24th and 48th hours. Values are expressed as mean ± SEM. Control group (no treatment, only 0.1% DMSO was used as a solvent). Irinotecan administered groups IR50 (50mM) and IR100 (100mM); Borax administered groups B1 and B3 (at concentrations of 1 mg/ml and 3 mg/ml, respectively). δ shows a significant difference from the 24th hours of each group. ($p<0.05$); *shows significantly difference from the 24th hours of Control ($p<0.05$); #shows significantly difference from the 48th hours of Control ($p<0.05$)

DOI: 10.5455/medscience.2022.11.253

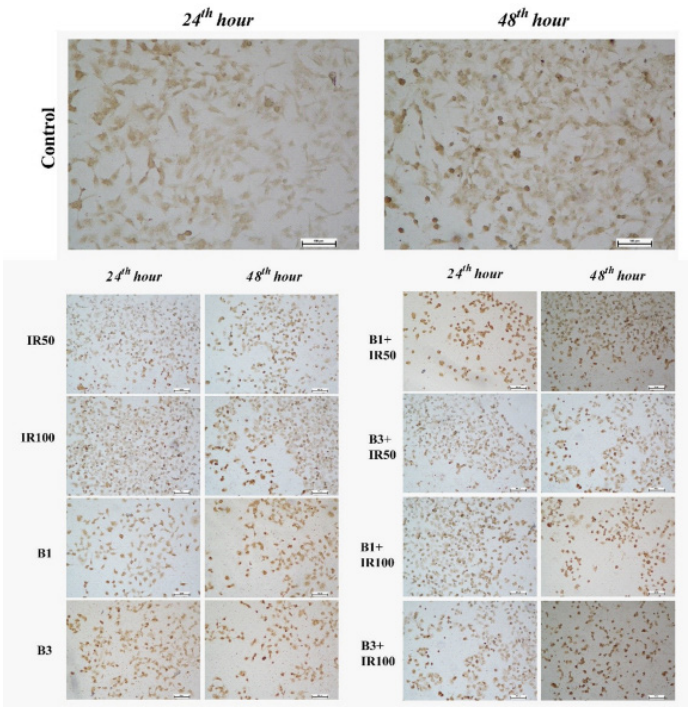

Figure 3. The immunohistochemically stained YKG1 glioblastoma cells (Tdt-TUNEL based apoptosis detection kit). The cells green in color are non-apoptotic and brown in color are apoptotic cells (×200, scale bar: 50 µm). Control group (no treatment, only 0.1% DMSO was used as a solvent). Irinotecan administered groups IR50 (50mM) and IR100 (100mM); Borax administered groups B1 and B3 (at concentrations of 1 mg/ml and 3 mg/ml, respectively)

Table 1. Effects of irinotecan and borax on YKG1 glioblastoma cells' viability after 24 and 48-hours of treatment

Groups	Cell viability (% of control)	
	24th hour	48th hour
Control	100	100
IR50	86±12	79±9.2
IR100	89±6.7	75±6.6
B1	73±8.4	54±6.3
B3	63±6.4	50±3.4
B1 + IR50	49±4.9	43±4.2
B3 + IR50	68±3.8	50±4.1
B1 + IR100	52±4.4	44±4.7
B3 + IR100	51±6	43±3.9

Values are expressed as mean ± SEM. Control group (no treatment, only 0.1% DMSO was used as a solvent). Irinotecan administered groups IR50 (50mM) and IR100 (100mM); Borax administered groups B1 and B3 (at concentrations of 1 mg/ml and 3 mg/ml, respectively)

Discussion

Brain tumors are very diverse in their biological behavior and therefore their treatments are considered a major issue in modern medicine. GBM is the most encountered primary cancer of the central nervous system in adults, characterized by uncontrolled, aggressive cell proliferation, infiltrative growth in the brain, and general resistance to conventional treatment [20,21]. Despite management improvements, the outcome of patients remains extremely poor, with a mean life expectancy of approximately 1-2 years. Different combination chemotherapies are considered to overcome this aggressive cancer. It has become clear that therapeutic improvements will likely depend on effective combination therapies targeting multiple mechanisms. More effective new agents, or novel synergic combinations, are required for GBM treatment [2,5-7]. Although temozolomide is the standard chemotherapy regimen for primary GBMs, recurrent GBMs are frequently treated with high doses of DNA topoisomerase inhibitors such as irinotecan [8,9].

Irinotecan, which inhibits Topoisomerase-I, is widely used as a second-line drug for the treatment of GBM. DNA topoisomerases are nuclear enzymes that reduce torsional stress in supercoiled DNA, allowing for selected regions of DNA to become sufficiently relaxed to permit its replication, recombination, repair, and transcription [23]. Irinotecan is broadly used in solid cancer therapy, especially in combination with other drugs [10,19]. In previous studies, topoisomerase inhibitors have demonstrated antitumor activity in human glioblastoma cell-line with multidrug resistance [24,25]. Irinotecan has the capacity to deactivate the proliferation of tumor cells by attenuating the early phase of the cell cycle, G1. At the same time, irinotecan can impede metastatic processes and induce apoptotic cell death which is primarily responsible for its anti-tumor activity [26]. SN-38 (7-Ethyl-10-hydroxycamptothecin) is a metabolite that occurs with the carboxylesterase-mediated breakdown of irinotecan and it is 100–1.000 times more potent than irinotecan. Glioma cells are capable of converting irinotecan to SN-38 by intrinsic tumor carboxylesterases [27,28].

The effects of boron on many diseases have been the subject of research in the field of health recently. The biological, medical, and environmental roles of boron have attracted considerable attention over the years. Boron is an essential element for normal growth and development which is involved in a series of important physiological functions, including membrane integrity, cell wall formation, calcium uptake, protein metabolism, nucleic acid metabolism, and translocation [11]. Furthermore, boron has extensively been used in a variety of industries, including insecticides, food preservatives, fire retardants, glass products, detergents, and reagents for chemical synthesis [14]. Because boron is preferably used in industry, its medical applications are omitted today. What impacts it may create on cancer patients has been one of the recent issues recently. The consumption of nutrients with rich boron contents such as nuts, avocados, broccoli, and raisins has been reported to decrease the risk of certain cancers. Dietary boron intake is associated with reduced some cancers risks such as prostate and lung cancers [15-18]. In addition, epidemiological, animal, and cell culture studies have identified boron compounds have anti-tumor properties as chemo-preventative agents [14]. It has been observed in a study

DOI: 10.5455/medscience.2022.11.253

that when administered to the cancer cells in the culture medium, different boron products have decelerated the proliferation of cancer cells. It has been shown that boron reduces the expression of cyclin proteins (cyclin A1, B1, C, D1, and E) which are effective in the cell cycle, inhibits Ca+ release by NAD+CADPR system, and induces apoptosis [29]. In light of this information, it has been considered that boron can show beneficial effects on cancer treatment. Accordingly, in an effort to find better treatments for glioblastoma, we tested and compared single borax and irinotecan and also combined borax + irinotecan agents for their ability to enhance the standard cytotoxic drug currently used to treat glioblastoma. Current literature sources indicate the predominant activity of irinotecan on GBM. In patients with recurrent glioblastoma, irinotecan has been found to significantly improve survival when combined with Bevacizumab [30]. In the cell culture study, it was stated that irinotecan alone or in combination with alisertib showed efficacy by providing strong inhibition of O6-methylguanine DNA methyl transferase. In the study, it was determined that irinotecan showed absolute synergy with other agents [31]. Another study's data reveal that the metformin and irinotecan have the potential to decrease neurons and U-87 MG glioblastoma cell viability, in vitro; however, in vivo studies disclose that the metformin and irinotecan have the capacity to reduce tumor size in Sprague–Dawley rats [32].

The present study demonstrated that irinotecan alone demonstrated more cytotoxicity on YKG1 cell-lines at 48th hour dose-dependently, as compared to 24th hour. Borax alone also showed higher cytotoxicity in both 24th and 48th hour than irinotecan alone administration. In addition, in borax-administered groups, an increase in cytotoxicity was observed from low dose groups to high dose groups in both 24th and 48th hours. We found that both borax and irinotecan, as a single agent, effectively induced apoptosis dose-dependently at 24th and 48th hours. The combination therapy of borax and irinotecan has additive cytotoxic effects on YKG1 cells. Combined use of borax and irinotecan treatment remarkably reduced cell proliferation on YKG1 glioblastoma cells compared to single treatments of them which appears to be a consequence of increased DNA damage, resulting in apoptosis induction. Combination of borax with irinotecan enhanced anti-tumor efficacy in vitro by inducing cell death by apoptosis, to impressively increase the efficacy of irinotecan, a topoisomerase-1 inhibitor used in cancer treatment [29]. Borax showed potent synergic effects with irinotecan, and the most potent combinations were shown in combinations of borax (1 mg/ml) + irinotecan (50 mM) at 24th hour and also borax (1 mg/ml) + irinotecan (50 mM), borax (1 mg/ml) + irinotecan (100 mM) and borax (3 mg/ml) + irinotecan (100 mM) combinations had remarkably strong synergistic cytotoxic and apoptotic effects to YKG1 cells at 48th hour. The data obtained indicate that borax potentiates the cytotoxic effects of ironotecan on YKG1 glioblastoma cells.

The obtained results show that individual use of borax and irinotecan demonstrated lower cytotoxic and apoptotic effects compared to the combined use. Nevertheless, these effects were observed more than in particular at high dose usages. The obtained results showed us that supplementation appropriate doses of borax can enhance the cytotoxic and apoptotic effects of irinotecan during irinotecan chemotherapy. Our data highlight the therapeutic potential of borax used in combination with the known chemotherapy drug irinotecan and offer important insights for the development of more effective and selective therapies against GBM. However, these dosages must be supported by phase 2 study levels, before the implementation of phase 3 of a large study.

Conclusion

It was found that irinotecan and borax as a single agent inhibited the proliferation of YKG1 cells and enhanced cytotoxicity and apoptosis. The irinotecan-borax combined therapy had a synergistic effect on the antitumor efficacy of glioblastoma treatment, not only increasing tumor cell apoptosis but also significantly inhibiting tumor cell proliferation. In patients with glioblastoma, the combination of borax and irinotecan may be promising for a new anti-cancer therapy. The effect of borax on mechanisms can be better understood if new studies are performed with different timings and doses with further investigations. However, more studies are needed to prove this synergism with the borax-irinotecan combination.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
This study were financed by Afyon Kocatepe University Scientific Research Commission (14.HIZ.DES.0 3)

Ethical approval
There is no need to get informed consent of ethics within the scope of this study.

References

1. Xu T, Chen J, Lu Y, Wolff JE. Effects of bevacizumab plus irinotecan on response and survival in patients with recurrent malignant glioma: a systematic review and survival-gain analysis. BMC Cancer. 2010;10:252.
2. Mesti T, Moltara ME, Boc M, et al. Bevacizumab and irinotecan in recurrent malignant glioma, a single institution experience. Radiol Oncol. 2015;49:80-5.
3. Tate MC, Aghi MK. Biology of angiogenesis and invasion in glioma. Neurotherapeutics. 2009;6:447-57.
4. Grauer OM, Wesseling P, Adema GJ. Immunotherapy of diffuse gliomas: biological background, current status and future developments. Brain Pathol. 2009;19:674-93.
5. Pektas MB, Aslan, E, Firat F, et al. Suppressive effects of resveratrol on epithelial-mesenchymal transition in the YKG1 glioblastoma cell-line. Med Sci. 2021;1:1293-8.
6. Robins HI, Chang S, Butowski N, Mehta M. Therapeutic advances for glioblastoma multiforme: current status and future prospects. Curr Oncol Rep. 2007;9:66-70.
7. Perry A, Schmidt RE. Cancer therapy-associated CNS neuropathology: an update and review of the literature. Acta Neuropathol. 2006;111:197-212.
8. Buckner JC, Brown LD, Cascino TL, et al. Phase II evaluation of infusional etoposide and cisplatin in patients with recurrent astrocytoma.

J Neurooncol. 1990;9:249-54.

9. Jeremic B, Grujicic D, Jevremovic S, et al. Carboplatin and etoposide chemotherapy regimen for recurrent malignant glioma: a phase II study. J Clin Oncol. 1992;10:1074-7.
10. Vredenburgh JJ, Desjardins A, Reardon DA, Friedman HS. Experience with irinotecan for the treatment of malignant glioma. Neuro Oncol. 2009;11:80-91.
11. Lu YB, Qi YP, Yang LT, et al. Boron-deficiency-responsive microRNAs and their targets in Citrus sinensis leaves. BMC Plant Biol. 2015;15:271.
12. Benderdour M, Bui-Van T, Dicko A, Belleville F. In vivo and in vitro effects of boron and boronated compounds. J Trace Elem Med Biol. 1998;12:2-7.
13. Naghii MR, Darvishi P, Ebrahimpour Y, et al. Effect of combination therapy of fatty acids, calcium, vitamin D and boron with regular physical activity on cardiovascular risk factors in rat. J Oleo Sci. 2012;61:103-11.
14. Usuda K, Kono K, Dote T, et al. An overview of boron, lithium, and strontium in human health and profiles of these elements in urine of Japanese. Environ Health Prev Med. 2007;12:231-7.
15. Institute of Medicine (US) Panel on Micronutrients. dietary reference intakes for vitamin a, vitamin k, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington (DC): National Academies Press (US); 2001
16. Barranco WT, Eckhert CD. Boric acid inhibits human prostate cancer cell proliferation. Cancer Lett. 2004;216:21-9.
17. Henderson KA, Kobylewski SE, Yamada KE, Eckhert CD. Boric acid induces cytoplasmic stress granule formation, eIF2α phosphorylation, and ATF4 in prostate DU-145 cells. Biometals. 2015;28:133-41.
18. Gallardo-Williams MT, Chapin RE, King PE, et al, Maronpot RR. Boron supplementation inhibits the growth and local expression of IGF-1 in human prostate adenocarcinoma (LNCaP) tumors in nude mice. Toxicol Pathol. 2004;32:73-8.
19. Nakatsu S, Kondo S, Kondo Y, et al. Induction of apoptosis in multi-drug resistant (MDR) human glioblastoma cells by SN-38, a metabolite of the camptothecin derivative CPT-11. Cancer Chemother Pharmacol. 1997;39:417-23.
20. Soysal Y, Tosun M, Uysal M, Yurtçu E. Borax potentiate the cytotoxic effect of paclitaxel on MCF-7 breast cancer cell-line. National Med Biol Gen Congr. 2013, Turkey.
21. Frosina G. Limited advances in therapy of glioblastoma trigger re-consideration of research policy. Crit Rev Oncol Hematol. 2015;96:257-61.
22. Alifieris C, Trafalis DT. Glioblastoma multiforme: Pathogenesis and treatment. Pharmacol Ther. 2015;152:63-82.
23. Gupta M, Fujimori A, Pommier Y. Eukaryotic DNA topoisomerases I. Biochim Biophys Acta. 1995;1262:1-14.
24. Singh S, Dwarakanath BS, Lazar Mathew T. Role of topoisomerases in cytotoxicity induced by DNA ligand Hoechst-33342 and UV-C in a glioma cell line. Indian J Exp Biol. 2005;43:313-23.
25. Hare CB, Elion GB, Houghton PJ, et al. Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against pediatric and adult central nervous system tumor xenografts. Cancer Chemother Pharmacol. 1997;39:187-91.
26. Whitacre CM, Zborowska E, Willson JK, Berger NA. Detection of poly(ADP-ribose) polymerase cleavage in response to treatment with topoisomerase I inhibitors: a potential surrogate end point to assess treatment effectiveness. Clin Cancer Res. 1999;5:665-72.
27. Kawato Y, Aonuma M, Hirota Y, et al. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res. 1991;51:4187-91.
28. Slatter JG, Su P, Sams JP, et al. Bioactivation of the anticancer agent CPT-11 to SN-38 by human hepatic microsomal carboxylesterases and the in vitro assessment of potential drug interactions. Drug Metab Dispos. 1997;25:1157-64.
29. Nielsen FH. Biochemical and physiologic consequences of boron deprivation in humans. Environ Health Perspect. 1994;102:59-63.
30. Cloughesy TF, Filka E, Nelson G, et al. Irinotecan treatment for recurrent malignant glioma using an every-3-week regimen. Am J Clin Oncol. 2002;25:204-8.
31. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009;27:4733-40.
32. Sak M, Zumbar CT, King PD, et al. Cytotoxic synergy between alisertib and carboplatin versus alisertib and irinotecan are inversely dependent on MGMT levels in glioblastoma cells. J Neurooncol. 2019;143:231-40.