Research article

Effects of resveratrol and 1,3-bis(2-chloroethyl)-1-nitrosurea combination on YKG1 glioblastoma cells

Gökhan PEKTAŞ[1]*, **Esra ASLAN**[2], **Hilal GÜZEL**[3], **Betül DEMİRCİLER YAVAŞ**[4], **Sefa ÇELİK**[5]
[1]*Muğla Sıtkı Koçman University, Faculty of Medicine, Department of Hematology, 48000, Mugla, Turkey*
[2]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, 03200, Afyonkarahisar, Turkey*
[3]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy, 03200, Afyonkarahisar, Turkey*
[4]*Private Practice, Traditional and Complementary Treatment Center, 03200, Afyonkarahisar, Turkey*
[5]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, 03200, Afyonkarahisar, Turkey*
[1]gokhanpektas@gmail.com, [2]dr_esragul@hotmail.com, [3]hilalgzl@hotmail.com, [4]betuldy@gmail.com, [5]sefa_celik@hotmail.com

Resveratrol ve 1,3-bis(2-kloroetil)-1-nitrozüre kombinasyonunun YKG1 glioblastoma hücreleri üzerine etkileri

Abstract: Glioblastoma is a primary malignant brain tumor that can be treated with 1,3-bis(2-chloroethyl)-1-nitrosurea (BiCNU/carmustine). Resveratrol is a natural phenol that can interfere with apoptosis. This study aims to investigate how the combination of BiCNU and resveratrol affects glioblastoma cells in vitro. Accordingly, YKG1 glioblastoma cells were treated with different amounts of resveratrol (50 and 100 µM) and BiCNU (10 and 20 µM) either alone or in combination. Cell viability tests and immunochemical studies were conducted on these cells. According to results, increasing the amount of resveratrol and BiCNU decreased cell viability. Additionally, when these maximal doses of resveratrol and BiCNU (100 µM resveratrol plus 20 µM BiCNU) were applied, viability decreased to the highest cytotoxicity levels. Immunohistochemical analysis also revealed the significantly upregulated H scores of beclin-1 and caspase-3 in treated groups with the highest value in maximally combined concentration. These results indicated the cumulative effects of concurrent administration of BiCNU and resveratrol on the cytotoxicity of malignant human YKG1 glioblastoma cells in vitro.

Keywords: Apoptosis, carmustine, glioblastoma, resveratrol

Özet: Glioblastoma, 1,3-bis (2-kloroetil) -1-nitrosüre (BiCNU / carmustin) ile tedavi edilebilen birincil kötü huylu beyin tümörüdür. Resveratrol, apoptozu engelleyebilen doğal bir fenoldür. Bu çalışma, BiCNU ve resveratrol kombinasyonunun glioblastoma hücrelerini in vitro nasıl etkilediğini araştırmayı amaçlamaktadır. Çalışma kapsamında glioblastoma YKG1 hücreleri, tek başına veya kombinasyon halinde farklı miktarlarda resveratrol (50 ve 100 uM) ve BiCNU (10 ve 20 uM) ile muamele edildi. Bu hücreler üzerinde hücre canlılığı testleri ve immünokimyasal çalışmalar yapıldı. Sonuçlara göre, resveratrol ve BiCNU miktarının artırılması hücre canlılığını kademeli olarak azalttı. Ek olarak, maksimum resveratrol ve BiCNU dozu (100 µM resveratrol artı 20 µM BiCNU) verildiğinde, hücre canlılıkları oldukça azaldı. İmmünohistokimyasal analizler, maksimum kombine konsantrasyonda muamele edilen gruplarda beklin-1 ve kaspaz-3 H skorları düzeylerini önemli ölçüde arttırdığını ortaya çıkarmıştır. Bu sonuçlar, BiCNU ve resveratrolün aynı anda uygulanmasının, malign insan YKG1 glioblastoma hücrelerinin in vitro sitotoksisitesi üzerindeki kümülatif etkilerini göstermiştir.

Anahtar Kelimeler: Apoptozis, karmustin, glioblastoma, resveratrol

1. Introduction

The brain's primary malignant tumor, glioblastoma (GB), is considered the second most frequently encountered brain tumor following the meningioma (Omuro and De Angelis, 2013). It has been reported that GB makes up 12% to 15% of all intracranial tumors and 50% to 60% of astrocytic tumors. This tumor occurs in 3 out of 100.000 individuals per year (Williams, 2014). It has been stated that GB often starts at around 64 years of age, and incidence in males was more common than females (Tian et al., 2018).

GB presents non-specific symptoms, including headache, nausea, vomiting, seizures, changes in personality, and symptoms resembling those of a stroke initially. These symptoms might become worsen rapidly and give rise to unconsciousness (Alexander and Cloughesy, 2017). The causes of GB is unknown in most cases. However, genetic disorders such as neurofibromatosis, Li Fraumeni syndrome and previous radiotherapy might pose risk factors for GB, which might originate from normal neurons or develop from an existing low-grade astrocytoma (Alifieris and Trafalis, 2015). Its diagnosis is based on computational tomography, magnetic resonance imaging, and tissue biopsy (Omuro and De Angelis, 2013; Batash et al., 2017).

Glioblastoma is a neurological pathology with high morbidity and mortality; therefore, its treatment is challenging for clinicians (Adamson et al., 2009). The first reason for this challenge is the susceptibility of brain damage from conventional treatments, and the second is the brain's limited capacity for repairing itself. The third reason is the resistance of the tumor cells to the traditional treatment methods, and the last is the inability of chemotherapeutics to cross the blood-brain barrier (Adamson et al., 2009; Batash et al., 2017).

The cure for GB refers to palliative treatment and other methods that intend to improve patients' survival. These methods consist of surgery, chemotherapy, radiotherapy, and immunotherapy (Alifieris and Trafalis, 2015). Thus, treatment of GB usually involves surgery followed by chemotherapy and radiotherapy. It is well known that more extensive surgical removal is associated with more prolonged survival (Villà et al., 2014). Chemotherapy for GB indicates the administration of vincristine, hydroxyurea, temozolomide, 6-thioguanine, 5-fluorouracil, and 1, 3-bis (2-chloroethyl)-1-nitrosurea (BiCNU) (Desai et al., 2019). BiCNU has been used both for the initial diagnosis of glioma and tumor recurrence via intravenous administration (Xiao et al., 2020). Amongst these, BiCNU which is an alkylating agent called carmustine, has been well established but may cause hepatotoxicity and pulmonary fibrosis (Desai et al., 2019). However, some studies have suggested that BiCNU provides a survival advantage for GB patients (Spiegel et al., 2007). Despite the overall treatment methods, GB usually remits, and the typical survival length following diagnosis is 12 to 15 months. Fewer than 7% of people might survive longer than five years, and without any treatment, the survival period is typically three months (Stoyanov et al., 2018; Witthayanuwat et al., 2018).

Apoptosis is a form of programmed cell death that occurs in multicellular organisms. In contrast to necrosis, which addresses traumatic cell death associated with acute cellular injury, apoptosis is an extremely controlled and regulated process that confers advantages during an organism's life cycle. These advantages especially appear during embryogenesis (Kaczanowski, 2016). Its high rate causes atrophy, but reduced apoptosis might cause uncontrolled cell proliferation, leading to carcinogenesis (Lopez and Tait, 2015; Kaczanowski, 2016).

A natural polyphenol, resveratrol (3, 5, 4'-trihydroxy-trans-stilbene), is produced by grapes, blueberries, raspberries, mulberries, and peanuts naturally (Kataria and Khatkar, 2019). It acts through signaling pathways related to growth factors and receptor tyrosine kinases and interferes with signal transduction by the growth factor β. Experimental studies have demonstrated that resveratrol might impact apoptosis by regulating molecules that comprise caspase-3, p53, peroxisome proliferator-activated receptor, NfκB, Bax, Bcl-2, and apoptotic protease activating factor-1 (Emsen and Turkez, 2017; Rauf et al., 2018).

Chemoprevention is defined as the administration of a pharmacological agent to prevent infection or disease. In malignancy, chemoprevention is initiated to avoid the spread of an existing condition, help differentiate molecular targets, and increase chemotherapeutics' bioavailability (Crusz and Balkwill, 2015). The use of chemoprophylaxis is limited primarily by two factors which are biological risk and financial cost. Since all medications can cause side effects, chemoprevention should only be considered when treatment benefits outweigh the risks. The cost associated with chemoprevention may be prohibitive, mainly when the cost of treatment is high or the target disease's incidence is low (Huang and Mellor, 2014). Resveratrol appears as an appropriate pharmacological for the chemoprevention due to its mild adverse effects and relatively low cost. Its adverse impacts are usually related to long-term use

and/or ingestion of higher doses. Nausea, stomach pain, flatulence, and diarrhea are the side effects of resveratrol treatment (Vervandeur-Fasser and Latruffe, 2014). The treatment process is very limited in GB, and there is no effective treatment. Therefore, this study aims to compare the effectiveness of resveratrol as an alternative to BiCNU treatment in the YKG1 glioblastoma cell line and to determine whether it potentiates the effects of each other depending on their possible combination.

2. Materials and Method

YKG1 human glioblastoma cells were obtained commercially (RIKEN BioResource Center, Japan), whereas analytical grade BiCNU (15493-8, SigmaAldrich, USA) and trans-resveratrol (CAS 501-36-0, Santa Cruz, USA) were used for pharmacological treatment.

2.1. Cell Culture

Cells were incubated in culture media, including Dulbecco's Modified Medium with 10% Fetal Calf Serum, 1mM sodium pyruvate, and 2 mM L-glutamine in an incubator with 5% CO_2 and 95% humidity at 37°C. Cells were grown in flasks of 75 cm^2 at an approximate density of $4 \times 10^4/cm^2$. After trypsinization, the cell suspension was transferred to sterile capped tubes and centrifuged at 400g and 25°C for 5 minutes. The cell pellet was mixed with new media that had a volume corresponding to 1/3 of the tube. Twenty µl of this suspension was mixed with 90 µl buffer solution (PBS + %1 FCS) and 100 µl trypan blue in an eppendorf tube. White-colored living cells were counted by a hemocytometer in microscopic examination. When the cell count became sufficient, they were passaged at a ratio of 1:4, allocated into new flasks, and allowed to replicate under convenient incubation conditions. Cell viability was assessed in samples obtained from these culture media.

2.2. Experimental Design

Malignant YKG1 human glioblastoma cells that were passaged and replicated in the laboratory were treated by different doses of BiCNU and resveratrol for 48-h. DMSO (0.1%) was the solvent for the chemicals, and all treatments are summarized in Table 1. All groups contained at least triple biological replicates.

Table 1. Different experimental applications on glioblastoma cells

Group 1	50 µM resveratrol
Group 2	100 µM resveratrol
Group 3	10 µM BiCNU
Group 4	20 µM BiCNU
Group 5	50µM resveratrol + 10 µM BiCNU
Group 6	50µM resveratrol + 20 µM BiCNU
Group 7	100 µM resveratrol + 10 µM BiCNU
Group 8	100 µM resveratrol + 20 µM BiCNU
Group 9	Control (0.1% DMSO)

2.3. Cell Viability Measurement

MTT (3-(4,5-methylthiazol-2-yl)-2,5-diphenyl tetrazolium -bromide) is a colorimetric agent used to access cell viability in vitro. Living cells degrades MMT enzymatically, leading to a color change. This experiment aims to determine the percentage of cells that keep their viability with respect to the control after pharmacologic

agents' treatment. According to the method, after 48 hours of incubation with different amounts of resveratrol and BiCNU alone or in combination, the cells were treated with 20 µl MTT dye (5 mg/ml) for 2 hours. Afterward, MTT was eliminated, and 200 µl DMSO was added to each well. Following an incubation period of 10 minutes, the color change was assessed at a wavelength of 540 nm. Cell viability was accepted as 100% in the control group.

2.4. Immunocytochemistry

Human glioblastoma cells were cultured in 12-well chamber slides, and these cultures were treated by pharmacological agents. At the 48th hour of pharmacological treatment, the growing media were removed, and the cells were fixed by 4% paraformaldehyde. After washing by phosphate buffer saline (PBS), they were put on ice floating in a 0.1% Triton-X100 solution for 15 minutes, and endogenous activation of peroxidation was provided by 3% H_2O_2 for 10 minutes. The cells were washed in PBS three times in 5 minutes and then kept in a blocking solution for 10 minutes. Later, the cells were incubated with primary antibodies of Beclin-1 (ab62472, 1/200) ve Caspase-3 (Sc-56053, 1/200) at room temperature for 1 hour, and the suspensions were washed by PBS three times. After the cells were treated with secondary antibodies, they were incubated with biotin for 30 minutes. Then, the cells were washed by PBS three times in 5 minutes and incubated with avidin for 30 minutes. After the cells were washed by PBS three times in 5 minutes, they were colored by 3-amino-9-ethyl carbazole. Finally, they were washed with distilled water and stained with Mayer's hematoxylin. The cells were counted by a light microscopy under X20 magnification by the Image Analysis Program (NIS, Japan). The percentage of stained cells was specified semi-quantitatively depending on the following score formula.

Score = (Staining scale+1) x (Percentage of cells stained at each scale)

Staining was designated as 0 (no staining), +1 (weak staining), +2 (moderate staining), and +3 (strong staining).

2.5. Statistical Analysis

Data were analyzed by SPSS (Statistical Package for Social Sciences) software version 22.0 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test analyzed the distribution of data. Continuous variables were expressed as mean ± standard deviation, and categorical variables were denoted as numbers or percentages where appropriate. Student t-test was used, and P-values less than 0.05 were accepted as significant.

3. Results

3.1. Viability Test Results

Figure 1 indicates the viability test results obtained after 48 hours of pharmacological treatment. Accordingly, cell cytotoxicity was significantly higher in Group 2 (cells treated with 100 µM resveratrol) than Group 1 (cells treated with 50 µM resveratrol). Similarly, cytotoxicity was significantly increased in Group 4 (cells treated with 20 µM BiCNU) than Group 3 (cells treated with 10 µM BiCNU). Compared to other groups, cytotoxicity was

significantly higher in Group 8 (cells treated with 100 µM resveratrol + 20 µM BiCNU).
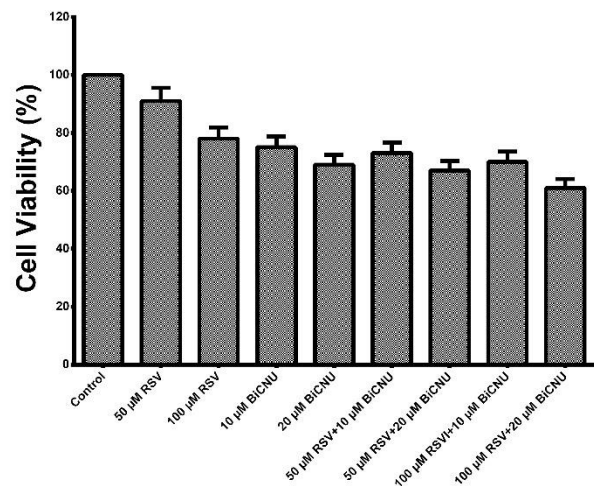

Figure 1. Effects of resveratrol (RSV) and 1,3-bis(2-chloroethyl)-1-nitrosurea (BiCNU) treatment on YKG1 glioblastoma cells' viability after 48-hours of treatment.

3.2. Immunocytochemistry Results

Expression levels of Beclin-1 and Caspase-3 proteins were analysed with immunohistochemical staining, and the results are summarised in Figure 2.
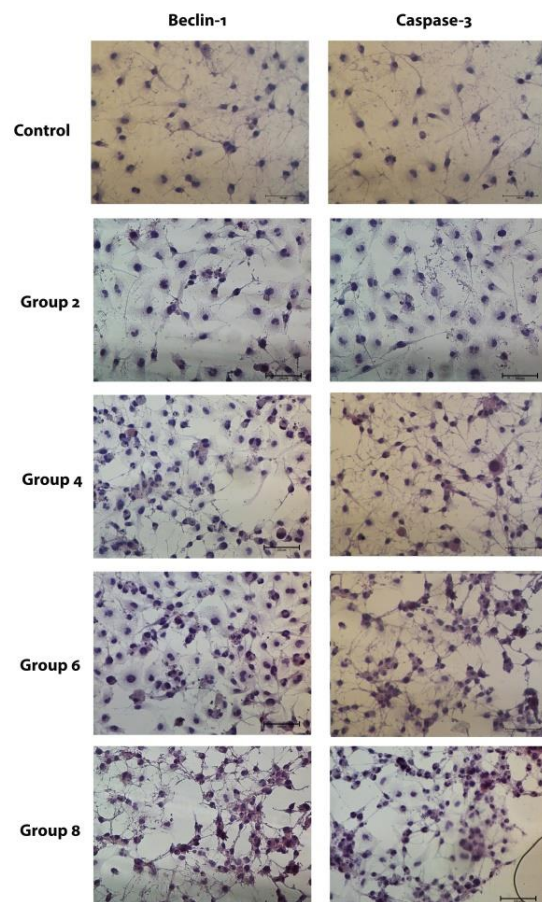

Figure 2. Images of the immunostained YKG1cells, which were treated with different amounts of resveratrol and BiCNU. The cells were stained with Beclin-1, and Caspase-3 antibodies and images were taken by a light microscopy at 20X magnification.

The images of immunostained slides were analysed semi-quantitatively, and Hscore results are summarized in Figure 3.
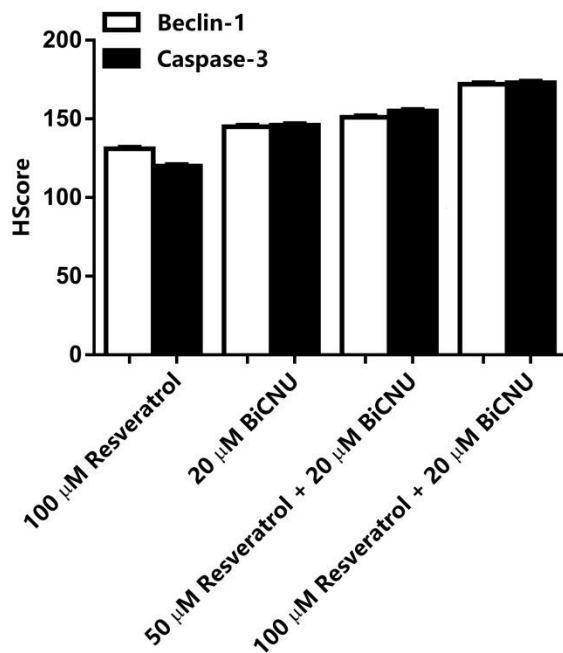

Figure 3. Beclin-1 and Caspase-3 HScore results obtained as a result of immunocytochemical staining.

According to results, the expression of Beclin-1 was significantly higher in Group 8 (cells treated with 100 μM resveratrol + 20μM BiCNU) as compared to Group 2, Group 4, Group 6 respectively (P < 0.0001, P =0.001, P= 0.002). The expression of Caspase-3 was significantly higher in Group 8 as compared to Group 2, Group 4, and Group 6 (P < 0.001, P <0.001 and P=0.004). All groups except Group 8 showed +1 immunostaining while there was both +1 and +2 immunostaining in Group 8 (cells treated with 100 μM resveratrol + 20 μM BiCNU).

4. Discussions

Glioblastoma is the most invasive tumor of the central nervous system with the worst prognosis among the other cancer types. The typical survival duration following diagnosis is around 12 to 15 months (Omuro and DeAngelis, 2013; Alexander and Cloughesy, 2017). The treatment of GB usually involves surgery followed by chemotherapy and radiotherapy. It is well known that larger surgical removal is associated with more prolonged survival (Villà et al., 2014; Alifieris and Trafalis, 2015). However, complete excision of GB is not possible in most cases, and the role of chemotherapy is often limited in preventing recurrences (Anjum et al., 2017). Therefore, an integrated approach should be developed to prolong the patients' survival span diagnosed with this malignancy.

Nitrosoureas, including BiCNU (carmustine), was first introduced to treat gliomas and their effectiveness was modest, but doses that produced response rates up to 50% leaded severe systemic side effects (Nagpal, 2012). Afterward, polymers were developed to maintain the efficacy of BiCNU beyond the blood-brain barrier so that, subsequently, biodegradable polymers might permit more constant drug delivery. Such BiCNU polymers were used to make up carmustine wafer which is also named as

BiCNU wafer or Gliadel wafer (Nagpal, 2012; Zhang et al., 2014).

The beneficial effects of carmustine wafer on patients' survival with GB have been highlighted in various studies. A meta-analysis found that treatment with carmustine wafer and temozolomide was significantly more effective than avoiding chemotherapy in improving survival. It was also remarked that the carmustine wafer's clinical efficiency extended up to 24 months (Spiegel et al., 2007). Another systematic review also aimed to examine the potency of carmustine wafer in malignant glioma treatment and included three randomized controlled trials and one prospective cohort study. This systematic review came up with the conclusion that the patients who received the diagnosis of GB for the first time and who were treated with carmustine wafer had a significantly longer survival span than the controls (Perry et al., 2007). Based on the findings of two randomized controlled trials, a Cochrane review concluded that carmustine wafer improved GB patients' survival without significantly contributing to the increase in adverse effects (Hart et al., 2011).

Resveratrol demonstrates many anti-carcinogenic effects on various cancer cells in vitro (Le Corre et al., 2005; Kundu and Surh, 2008; Shukla and Singh, 2011). When evidence from in vitro studies was analyzed, Jang et al. (1997) were the first showing resveratrol as a chemopreventive agent. It was found that topical application of resveratrol could inhibit tumor formation in an animal model of skin cancer. Later studies also confirmed that resveratrol's topical application prevented tumor formation by regulating the cell cycle and endorsing apoptosis, reducing COX activity and prostaglandin production in a skin cancer mouse model (Afaq et al., 2003; Reagan-Shaw et al., 2004). The findings related to in vitro use and efficacy of resveratrol for other types of cancer that require its oral ingestion or intraperitoneal injection has been more controversial. This discrepancy has been attributed to the poor bioavailability of trans-resveratrol. Wenzel & Somoza (2005) clarify the bioavailability and metabolism of resveratrol. Accordingly, as resveratrol is consumed orally in rodents and humans, 70–80% is quickly absorbed via passive diffusion in the intestines (Kaldas et al., 2003; Walle et al., 2004). After absorption, resveratrol is conjugated into glucuronides and sulfates so that trans-resveratrol levels in peripheral circulation reach their peak 30–60 minutes after oral administration (Soleas et al., 2001; Yu et al., 2002).

In humans, circulating unmodified trans-resveratrol levels make up only about 2% of the peak serum concentration of total free resveratrol and conjugates after a single dose of 25mg/70kg body weight (Goldberg et al., 2003). Another study has ended up with the conclusion that at least 70% of resveratrol is absorbed after a single 25mg dose, and there is a peak serum concentration of 2μM (approximately 490 ng/ml) for resveratrol and all of its metabolites (Walle et al., 2004). After administering multiple oral doses (5g daily for 29 days), plasma concentrations of trans-resveratrol are as high as 4 μM (4.29 nmol/ml). However, it should be noted that resveratrol at this high dose was also associated with gastrointestinal side effects (Brown et al., 2010). On the contrary, in human colon tissue, resveratrol levels and its

metabolite resveratrol-3-O-glucuronide have been detected in relatively higher concentrations (674 and 86 nmol/g, respectively) when 0.5–1.0 g of resveratrol was taken orally once per day. In the study mentioned above, resveratrol supplementation decreased cellular proliferation by 5% in colorectal cancer tissue, as assessed by Ki67 staining (Patel et al., 2010). Since there are such rapid conjugation and low bioavailability of resveratrol, the in vitro use of resveratrol for cancer prevention and treatment has been a matter of debate.

As for brain cancer, Xu and coworkers were the first reporting the combination of resveratrol, and temozolomide significantly down-regulates the expression of matrix metalloproteinase-9, enhances the production of reactive oxygen species, and inhibits the anti-apoptotic protein Bcl-2. Thus, this combination has been considered to suppress cell proliferation in malignant U87MG glioma cell line. The significant pro-apoptotic effect of resveratrol has been observed through the increase in Bax expression, the decrease in the expression of Bcl-2, and the cleavage of caspase-3 (Xu et al., 2005).

Filippi-Chiela and colleagues were the first to determine that resveratrol induced autophagy formation by the up-regulation of autophagy proteins such as Atg5, Beclin-1, and LC3-II in three human glioblastoma cell lines. Correspondingly, the authors have hypothesized that resveratrol accelerates autophagy inhibition, which results in apoptosis in turn (Filippi-Chiela et al., 2011). Firouzi and coworkers found that methoxyamine and resveratrol can significantly reduce colony numbers and induce DNA damage of glioblastoma spheroid cells. This result reflected the promise of resveratrol at a 20-μM concentration in cancer-treatment therapy when used together with radiation and radiosensitizer (Firouzi et al., 2015). Other researchers demonstrated that resveratrol might reduce the expression and activity of the POK erythroid ontogenic factor (Pokemon) in glioma cells, suppress the Sp1 DNA binding activity, and enhance the recruitment of HDAC1 (Yang et al., 2016). Later, Song et al. showed that administration of 20 μM resveratrol decreases cell viability to a lesser extent and the administration of 40 μM resveratrol decreases cell viability significantly in malignant human LN18 and U87 glioblastoma cell lines (Song et al., 2019). Another study by Cilibrasi et al. found out that resveratrol at a dose of 100 μM had the highest efficiency for cytotoxicity for human glioma stem cells. In that study, cytotoxicity was observed at the minimum resveratrol dose of 50 μM but, interestingly, the resveratrol dose of 200 μM was found to have significantly lower cytotoxicity than the resveratrol dose of 100 μM (Cilibrasi et al., 2017).

In this study, cytotoxicity was significantly higher in the cells treated with 100 μM resveratrol than the cells treated with 50 μM resveratrol. Likely, toxic effects was significantly increased in the cells treated with 20 μM BiCNU than the cells treated with 10 μM BiCNU. Compared to other groups, cell viability was significantly higher in the cells treated with 100 μM resveratrol + 20 μM BiCNU. Moreover, the expression of Beclin-1 and Caspase-3 were significantly higher in cells treated with 100 μM resveratrol + 20μM BiCNU than in the other groups. All groups except those treated with 100 μM resveratrol + 20 μM BiCNU showed +1 immunostaining while there was both +1 and +2 immunostaining in cells treated with 100 μM resveratrol + 20 μM BiCNU. The present study's findings suggest that the concurrent administration of BiCNU and resveratrol has cumulative effects on the cytotoxicity of malignant human YKG1 glioblastoma cells in cell culture. This cytotoxicity is related to both autophagy and apoptosis as it is reflected at least enhanced expression of Beclin-1 and Caspase-3. The present study's findings should be interpreted carefully as their power is limited by the in vitro design of the research and the administration of relatively lower doses of BiCNU and resveratrol to the cell culture. A clinical implication of these findings might be the utilization of resveratrol as a chemoprevention agent in patients receiving BiCNU to treat malignant glioma. To verify this implication, clinical studies should be undertaken to specify the efficacy and safety of concurrent BiCNU and resveratrol treatment in patients diagnosed with malignant glioma. Hence, further research is warranted to clarify the effects of the BiCNU and resveratrol combination for the glioblastoma cells in vitro and the GB patients in vitro.

Conflict of interest

The authors state no conflict of interest.

Author Contribution

GP, EA, HG, BDY, and SÇ performed the research. EA and HG helped during the experimental study and statistical analysis. GP wrote the manuscript. GP and EA drafted the manuscript. GP and EA conceived and designed the study and critically revised the manuscript.

Acknowledgments

References

Adamson C, Kanu OO, Mehta AI, Di C, Lin N, Mattox AK, Bigner DD (2009). Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opinion of Investig Drugs 18(8): 1061-1083.

Afaq F, Adhami VM, Ahmad N (2003). Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. Toxicology and Applied Pharmacology 186(1): 28-37.

Alexander BM, Cloughesy TF (2017). Adult Glioblastoma. Journal of Clinical Oncology 35(21): 2402-2409.

Alifieris C, Trafalis DT (2015). Glioblastoma multiforme: Pathogenesis and treatment. Pharmacology and Therapeutics 152: 63-82.

Anjum K, Shagufta BI, Abbas SQ, Patel S, Khan I, Shah SAA, Akhter N, Hassan SSU (2017). Current status and future therapeutic perspectives of glioblastoma multiforme (GB) therapy: A review. Biomedicine and Pharmacotherapy 92: 681-689.

Batash R, Asna N, Schaffer P, Francis N, Schaffer M (2017). Glioblastoma Multiforme, Diagnosis and Treatment; Recent Literature Review. Current Medicinal Chemistry 24(27): 3002-3009.

Brown VA, Patel KR, Viskaduraki M, Crowell JA, Perloff M, Booth TD, Vasilinin G, Sen A, Schinas AM, Piccirilli G, Brown K, Steward WP, Gescher AJ, Brenner DE (2010). Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. Cancer Research 70(22): 9003-9011.

Cilibrasi C, Riva G, Romano G, Cadamuro M, Bazzoni R, Butta V, Paoletta L, Dalprá L, Strazzabosco M, Lavitrano M, Giavannonni R, Bentivegna A (2017). Resveratrol impairs glioma stem cells proliferation and motility by modulating the wnt signaling pathway. PLoS One 12(1): e0169854.

Crusz SM, Balkwill FR (2015). Inflammation and cancer: advances and new agents. Natural Reviews Clinical Oncology 12(10): 584-596.

Desai K, Hubben A, Ahluwalia M (2019). The Role of Checkpoint Inhibitors in Glioblastoma. Targeted Oncology 14(4): 375-394.

Emsen B, Turkez H (2017). The protective role of resveratrol against zinc oxide induced nanotoxicity. Anatolian Journal of Botany 1(2): 21-25.

Filippi-Chiela EC, Villodre ES, Zamin LL, Lenz G (2011). Autophagy interplay with apoptosis and cell cycle regulation in the growth-inhibiting effect of resveratrol in glioma cells. PLoS One 6(6): e20849.

Firouzi F, Khoei S, Mirzaei HR (2015). Role of resveratrol on the cytotoxic effects and DNA damages of iododeoxyuridine and megavoltage radiation in spheroid culture of U87MG glioblastoma cell line. General Physiology and Biophysics 34(1): 43-50.

Goldberg DM, Yan J, Soleas GJ (2003). Absorption of three wine-related polyphenols in three different matrices by healthy subjects. Clinical Biochemistry 36(1): 79-87.

Hart MG, Grant R, Garside R, Rogers G, Somerville M, Stein K (2011). Chemotherapy wafers for high grade glioma. Cochrane Database Systematic Review 1(3): CD007294.

Huang L, Mellor AL (2014). Metabolic control of tumour progression and antitumour immunity. Current Opinion in Oncology 26(1): 92-99.

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275(5297): 218-220.

Kaczanowski S (2016). Apoptosis: its origin, history, maintenance and the medical implications for cancer and aging. Physical Biology 13(3): 031001.

Kaldas MI, Walle UK, Walle T (2003). Resveratrol transport and metabolism by human intestinal Caco-2 cells. Journal of Pharmacy and Pharmacology 55(3): 307-312.

Kataria R, Khatkar A (2019). Resveratrol in Various Pockets: A Review. Current Topics in Medicinal Chemistry 19(2): 116-22.

Kundu JK, Surh YJ (2008). Cancer chemopreventive and therapeutic potential of resveratrol: mechanistic perspectives. Cancer Letters 269(2): 243-261.

Le Corre L, Chalabi N, Delort L, Bignon YJ, Bernard-Gallon DJ (2005). Resveratrol and breast cancer chemoprevention: molecular mechanisms. Mol Nutrition and Food Research 49(5): 462-471.

Lopez J, Tait SW (2015). Mitochondrial apoptosis: killing cancer using the enemy within. British Journal of Cancer 112(6): 957-962.

Nagpal S (2102). The role of BCNU polymer wafers (Gliadel) in the treatment of malignant glioma. Neurosurgery Clinics of North America 23: 289-295.

Omuro A, DeAngelis LM (2013). Glioblastoma and other malignant gliomas: A clinical review. Journal of American Medical Association 310(17): 1842-1850.

Patel KR, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ, Brown K (2010). Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. Cancer Research 70(19): 7392-7399.

Perry J, Chambers A, Spithof K, Laperriere N (2007). Gliadel wafers in the treatment of malignant glioma: A systematic review. Current Oncology 14: 189-194.

Rauf A, Imran M, Butt MS, Nadeem M, Peters DG, Mubarak MS (2018). Resveratrol as an anti-cancer agent: A review. Critical Reviews in Food Science and Nutrition 58(9): 1428-1447.

Reagan-Shaw S, Afaq F, Aziz MH, Ahmad N (2004). Modulations of critical cell cycle regulatory events during chemoprevention of ultraviolet B-mediated responses by resveratrol in SKH-1 hairless mouse skin. Oncogene 23(30): 5151-5160.

Shukla Y, Singh R (2011). Resveratrol and cellular mechanisms of cancer prevention. Annals of the New York Academy of Sciences 1215: 1-8.

Soleas GJ, Angelini M, Grass L, Diamandis EP, Goldberg DM (2001). Absorption of trans-resveratrol in rats. Methods in Enzymology 335: 145-154.

Song Y, Chen Y, Li Y, Lyu X, Cui J, Cheng Y, Zheng T, Zhao L, Zhao G (2019). Resveratrol Suppresses Epithelial-Mesenchymal Transition in GB by Regulating Smad-Dependent Signaling. Biomed Research International 2019: 1321973.

Spiegel BM, Esrailian E, Laine L, Chamberlain MC (2007). Clinical impact of adjuvant chemotherapy in glioblastoma multiforme: A meta-analysis. Central Nervous System Drugs 21: 775-787.

Stoyanov GS, Dzhenkov D, Ghenev P, Iliev B, Enchev Y, Tonchev AB (2018). Cell biology of glioblastoma multiforme: from basic science to diagnosis and treatment. Medical Oncology 35(3): 27.

Tian M, Ma W, Chen Y, Yu Y, Zhu D, Shi J, Zhang Y (2018). Impact of gender on the survival of patients with glioblastoma. Bioscience Reports 38(6): BSR20180752.

Vervandier-Fasseur D, Latruffe N (2019). The Potential Use of Resveratrol for Cancer Prevention. Molecules 24(24): 4506.

Villà S, Balañà C, Comas S (2014). Radiation and concomitant chemotherapy for patients with glioblastoma multiforme. Chinese Journal of Cancer 33(1): 25-31.

Walle T, Hsieh F, DeLegge MH, Oatis JE Jr, Walle UK (2004). High absorption but very low bioavailability of oral resveratrol in humans. Drug Metabolism and Disposition 32(12): 1377-1382.

Wenzel E, Somoza V (2005). Metabolism and bioavailability of trans-resveratrol. Molecular Nutrition and Food Research 49(5): 472-481.

Williams DS (2014). Glioblastoma multiforme. Journal of Insurance Medicine 44(1): 62-64.

Witthayanuwat S, Pesee M, Supaadirek C, Supakalin N, Thamronganantasakul K, Krusun S (2018). Survival Analysis of Glioblastoma Multiforme. Asian Pacific Journal of Cancer Prevention 19(9): 2613-2617.

Xiao ZZ, Wang ZF, Lan T, Huang WH, Zhao YH, Ma C, Li ZQ (2020). Carmustine as a Supplementary Therapeutic Option for Glioblastoma: A Systematic Review and Meta-Analysis. In Frontiers in Neurology 11: 1036.

Xu GW, Mymryk JS, Cairncross JG (2005). Pharmaceutical-mediated inactivation of p53 sensitizes U87MG glioma cells to BCNU and temozolomide. International Journal of Cancer 116(2): 187-192.

Yang Y, Cui J, Xue F, Overstreet AM, Zhan Y, Shan D, Li H, Li H, Wang Y, Zhang M, Yu C, Xu ZD (2016). Resveratrol represses pokemon expression in human glioma cells. Molecular Neurobiology 53(2): 1266-1278.

Yu C, Shin YG, Chow A, Li Y, Kosmeder JW, Lee YS, Hirschelman WH, Pezzuto JM, Mehta RG, van Breemen RB (2002). Human, rat, and mouse metabolism of resveratrol. Pharmacological Research 19(12): 1907-1914.

Zhang YD, Dai RY, Chen Z, Zhang YH, He XZ, Zhou J (2014). Efficacy and safety of carmustine wafers in the treatment of glioblastoma multiforme: a systematic review. Turkish Neurosurgery 24(5): 639-645.