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Evaluation of the Antitumor Activity of Omipalisib, a PI3K/AKT/MTOR Pathway Inhibitor, on Burkitt Lymphoma Cell Line

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ABSTRACT

There are many challenges in the treatment of Burkitt lymphoma, especially in immunocompromised individuals, elderly patients, and patients with relapsed or refractory disease. Therefore, there is a need for new and less toxic therapeutic agents. The aim of this study was to determine the antitumoral activity of omipalisib, a PI3K/AKT/mTOR pathway inhibitor, in the Burkitt lymphoma. Raji cell line was used in the study. Omipalisib was administered to the cell line and then the cytotoxic effect of omipalisib on Raji cells was evaluated by the XTT test. The IC50 value was calculated according to the results of the XTT test. Apoptosis and cell cycle experiments were studied with the calculated IC50 value. The flow cytometric method was used to determine the effect of omipalisib on apoptosis and cell death. The results of the study showed a statistically significant cytotoxic effect of increasing concentrations of omipalisib on Raji cells. The apoptosis experiment performed revealed that omipalisib strongly induced apoptosis. The cell cycle experiment showed that omipalisib stimulated the cell cycle arrest at the G0/G1 phase. It was concluded that omipalisib exhibited antitumoral activity on Burkitt lymphoma cells with its cytotoxic effect and induced apoptosis and cell cycle arrest. Considering this effect, targeting the PI3K/AKT/mTOR pathway with omipalisib can be a new treatment option.

Keywords: Apoptosis, Cell cycle, Omipalisib, Raji cells, XTT.

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Introduction

Burkitt lymphoma (BL) is a highly aggressive type of Non-Hodgkin Lymphoma (NHL). It arises from mature germinal or post-germinal center B cells. BL causes c-Myc overexpression and is the fastest growing human tumor with a 24-48 hour doubling time [1]. There are 3 different clinical types of BL: sporadic BL, endemic BL, commonly seen in African children (EBV has been detected in the vast majority of such patients), and immunodeficiency-related BL, usually seen in HIV-infected individuals. It is most commonly seen in young adults and children [2]. Bone marrow and central nervous system involvement are also common [3]. About 70% of patients have an advanced stage at the time of diagnosis [2]. BL is characterized by a high proliferation index and high turnover due to a high apoptosis rate [4].

The PI3K/AKT/mTOR pathway is highly correlated with the development and function of normal lymphocytes. Activation of the PI3K/AKT pathway is associated with BL lymphomagenesis and modulates cellular processes such as cell growth, cell proliferation, survival, and increased cell migration, as well as dysregulated apoptosis and oncogenesis [5-7]. Activation of the PI3K/AKT/mTOR pathway is associated with many solid tumors and hematological malignancies [8]. Omipalisib (GSK2126458) is a very potent and selective inhibitor of PI3K and mTOR and has been clinically tested in many different solid tumors, which has been demonstrated to target the PI3K/AKT/mTOR pathway. Inhibition of these signals stimulates apoptosis in tumor cells and disrupts biochemical processes of the cell such as oxidative phosphorylation and cellular respiration. Activation of this pathway and key substrates in this pathway plays a role in the prevention of cell death. These signals are highly active in many types of cancer, and PI3K/AKT pathway genetic mutations are implicated in the pathogenesis of different human cancers [9]. This agent has been studied in many different solid tumors and lymphomas and is expected to be beneficial in different cancer types by inhibiting the PI3K/AKT/mTOR pathway [10].

The aim of this study was to evaluate the cytotoxic effect of omipalisib, which inhibits the PI3K/AKT/mTOR pathway, on Burkitt lymphoma cells as well as its effects on the cell cycle with its apoptosis-inducing effect, thus demonstrating the antitumoral activity of this drug and revealing the benefits it can add to the treatment of Burkitt lymphoma.

Materials and Methods

Cell Culture Techniques

CCL-86 coded Human Burkitt lymphoma Raji cell line obtained from ATCC (American Type Cell Collection) was used in the research. The approval for the conduct of the study was was obtained from the Ethics Committee of Non-Invasive Clinical Researches of Sivas Cumhuriyet University with the number of 2021-08/11 and date 19.08.2021.

Raji cells were grown by passage when they reached 80% density in 25 cm^2 flasks using cell culture medium mixtures obtained by adding 10% fetal bovine serum and 1% penicillin-streptomycin into a RPMI 1640 medium. Cell culture studies were carried out at 37°C in a humidified atmosphere with 5% CO_2.

XTT Cell Viability Assay

The effect of Omipalisib, which has been shown to affect the PI3-Kinase and mTOR pathway, on the viability of Raji cells was investigated by the XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) assay (Roche Diagnostic, Germany). The method is based on the principle that metabolically active cells reduce XTT, a tetrazolium salt, to orange formazan components. Although the dye formed is water-soluble, the dye density can be read at the given wavelengths with the help of a spectrophotometer. The dye intensity (orange color) is proportional to the number of metabolically active cells.

For cytotoxicity, $10x10^3$ cells were first placed in each well and planted in a sterile 96-well microplate. Afterward, omipalisib was administered alone to Raji cells at 50, 25, 12.5, 1, 0.1, 0.01 µM concentrations and incubated for 24 hours. At the end of 24 hours, 50 µl of XTT solution was added to each well and incubated for 4 hours in a CO_2 oven. At the end of the incubation period, the optical density (OD) value was read with a microplate reader at 450 nm, and the cell viability rate of the control group was considered 100%; the half-inhibitory concentration (IC_{50}) value was calculated using the following formula: % cell viability = (Concentration OD/Control OD) x 100. Apoptosis and cell cycle experiments were studied over the obtained IC_{50} values.

Flow Cytometric Analysis of Apoptosis and Cell Death

Annexin V is a calcium-dependent and phospholipid-binding protein with a high affinity to phosphatidylserine residues. In the early stages of apoptosis, phosphatidylserine residues translocate to the outer surface of the outer membrane so that they can be stained with annexin V dye. In our study, the Muse™ Cell Analysis System and the Muse™ Annexin V & Cell death kit (Merck, Millipore, USA), which is compatible with this system, were used to detect apoptosis and cell death. This kit not only stains phosphatidylserine residues of apoptotic cells with Annexin V dye but also evaluates the structural integrity of the cell membrane with a 7-ADD stain. The protocol is based on the direct detection of early apoptotic cells in culture. The final concentration of cells was adjusted to $2x10^4$-$1x10^5$ cells/well (or $1x10^5$ to $5x10^5$ cells/mL) for optimal results. The PI3K/mTOR inhibitor omipalisib at IC_{50} concentration was administered to Raji cells used in the study and incubated for 24 hours. At the end of 24 hours, the cells were centrifuged at 1000 x rpm for 5 minutes to obtain a cell pellet. Approximately 20 minutes after Annexin V dye was added to the pellet, it was read by the device.

Cell Cycle Analysis

Disturbances in cell cycle regulation are a characteristic feature of tumor cells, and mutations in genes under cell cycle control are extremely common in cancer formation. For these reasons, cell cycle analysis is very important to understand the mechanisms of action of anti-cancer compounds and to examine the mechanisms of cell division. In the present study, cell cycle analysis was performed using the Muse™ Cell Cycle Kit (Merck, Millipore, USA) in accordance with the kit procedure. Propidium iodide (PI) and RNAase A reagents intercalating to DNA were used for the analysis. In the presence of RNAase, the PI reagent distinguishes the different stages of the cell cycle depending on the difference in the DNA content of the cell. Cells at rest (G0/G1) contain two copies of each chromosome. When cells begin their division cycle, they synthesize chromosomal DNA (S phase). The fluorescence intensity from PI increases until all chromosomal DNA is doubled (G2/M phase). At this stage, cells in the G2/M stage fluoresce twice the intensity of the G0/G1 population. G2/M cells eventually divide into two cells. The test gives the percentage of cells in each cell cycle phase (G0/G1, S, and G2/M) separately thanks to PI-based staining of the DNA content. In this study, PI3K/mTOR inhibitor omipalisib at IC_{50} concentration was administered to Raji cells and incubated for 24 hours. At the end of 24 hours, the cells were centrifuged at 1000 x rpm for 5 minutes to obtain a cell pellet. Cells were fixed and prepared for analysis in accordance with the kit procedure. The cell pellet was resuspended with Muse Cell Cycle Kit™ reagent and then incubated for 30 minutes at room temperature by protecting from light. Cell population distribution at different stages of the cell cycle was determined using the Muse™ Cell Analyzer (Merck, Millipore, USA).

Statistical Analysis

Statistical analysis of the study data was carried out using the SPSS software package. One-way ANOVA (Analysis of Variance) test was used for normally distributed data, while the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed data. A $p\text{-value}<0.05$ was considered significant in all analyses.

Results

Cytotoxic Effect of Omipalisib on Raji Cells

Omipalisib was administered to BL Raji cells at concentrations of 50, 25, 12.5, 1, 0.1, and 0.01 µM. The cytotoxic effect of these doses was evaluated at 24 hours. Omipalisib was observed to have a strong cytotoxic effect in a concentration-dependent manner. The IC_{50} value produced by omipalisib at 24 hours was calculated to be 3.04 µM. A strong cytotoxic effect was observed at 1, 12.5, 25, and 50 µM concentrations ($p<0.01$). Although the omipalisib concentrations of 0.01 and 0.1 µM had cytotoxic effects, no statistically significant difference was observed ($p>0.05$). The cytotoxic effect of omipalisib was dose-dependent and stronger at increasing concentrations. Although significant cytotoxic effects were observed at 1 µM and subsequent doses, the cytotoxic effect became stronger, especially at 12.5 µM and later doses ($p<0.01$) (Figure 1).

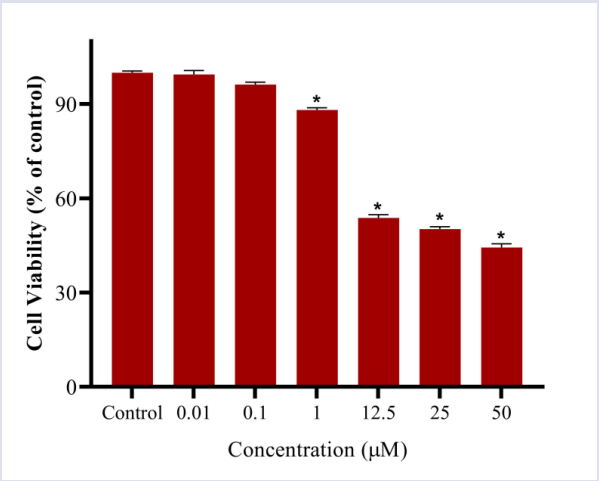


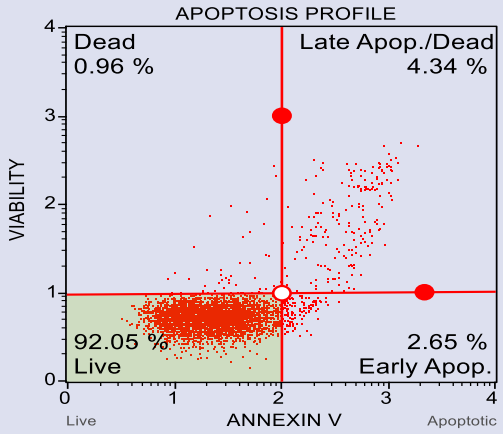
Figure 1. Evaluation of the Cytotoxic Effect of Omipalisib on Raji Cells at 24 Hours

* Statistically significant difference with the control ($p<0.01$).

Apoptotic Effect of Omipalisib on Raji Cells

The flow cytometric method was used to evaluate the apoptotic effects of omipalisib on Burkitt lymphoma Raji cells. There was a significant increase in apoptotic cells at IC50 concentration compared to the control group at 24 hours. Compared with the control group (4.34%), there was a significant increase in late apoptotic cells (31.35%), with a viable cell rate of 92.05% in the control group, while it was 63.02% in the omipalisib-treated group, with a statistically significant decrease ($p<0.01$). There was also an increase in the number of dead cells, but it was not statistically significant. While the rate of dead cells was 0.96% in the control group, it was 3.24% in the omipalisib group ($p>0.05$). A decrease was observed in the rate of early apoptotic cells, but it was not statistically significant. While the rate of early apoptotic cells was 2.65% in the control cell group, it was 2.39% in the omipalisib-treated cell group ($p>0.05$) (Figures 2 and 3). Omipalisib was found to induce apoptosis in Raji cells, and this property was shown to play an important role in its antitumoral activity.

Control



Omipalisib

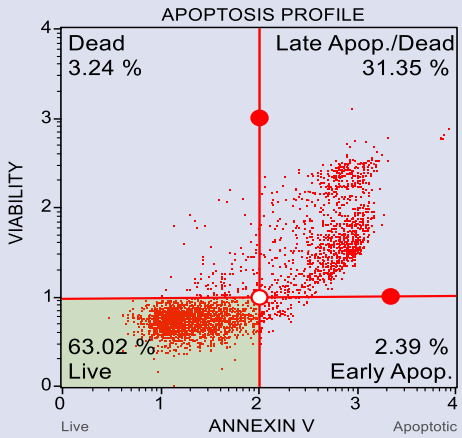


Figure 2. Quadrant Chart of Evaluation of Apoptotic Effect of Omipalisib on Raji Cells

Effect of Omipalisib on Cell Cycle of Raji Cells

The effect of Omipalisib on the cell cycle of Raji cells was investigated. Cells were treated with the IC50 concentration of omipalisib, and the effect was evaluated at 24 hours. The cell population accumulated in the G0/G1 phase in the omipalisib group, with a statistically significant difference with the control group ($p<0.01$) (Figure 4). Thus, it was determined that cell cycle arrest by omipalisib in the G0/G1 phase is another mechanism of action of its antitumoral activity.

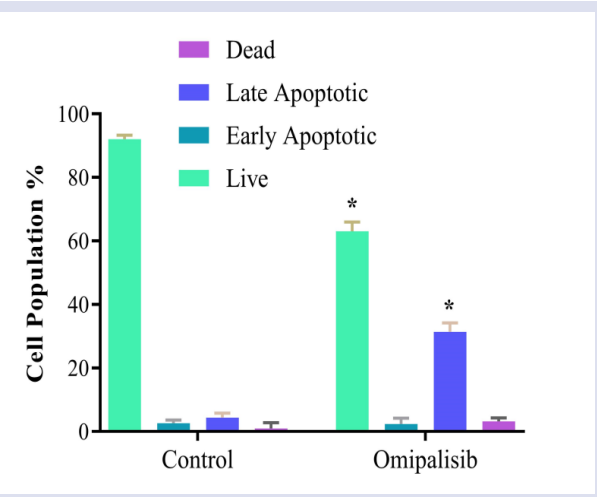
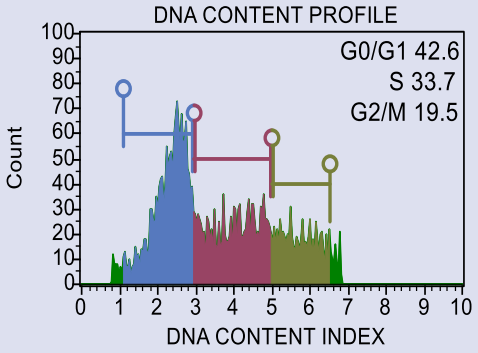


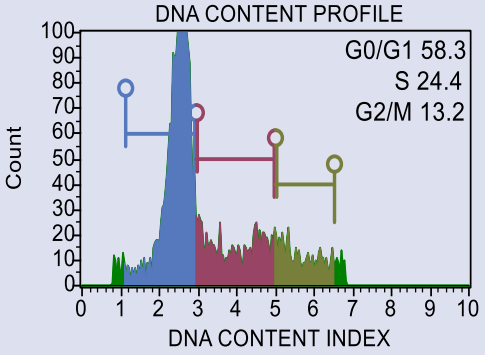
Figure 3. Bar Plot of Evaluation of Apoptotic Effect of Omipalisib on Raji Cells

* Statistically significant difference with the control (p<0.01).

Control



Omipalisib



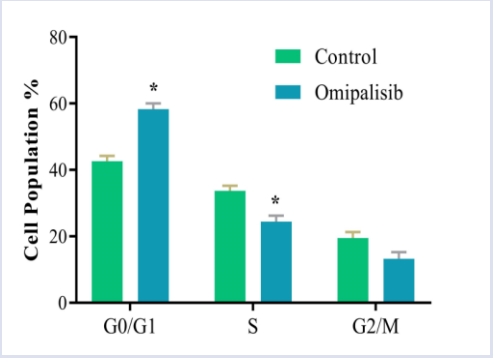


Figure 4. Evaluation of the Effect of Omipalisib on Cell Cycle of Raji Cells.

* Statistically significant difference with the control (p<0.01).

Discussion

Although BL is a very aggressive and fast-growing tumor, it is potentially curable with the use of combination chemotherapy regimens [11]. Regimes such as R-hyperCVAD, CODOX-M/IVAC, DA-EPOCH-R, and LMB are most commonly used for the treatment of BL [12]. Patients with relapsed/refractory BL have a very poor prognosis and often fail to achieve recovery with regimens [13,14]. With the emergence of new genomic findings for the treatment of BL, many new therapeutic agents have been under study. Activation of the PI3K/AKT/mTOR pathway is associated with the BL pathogenesis and poor prognosis in aggressive B-cell NHLs [5]. Ippolito et al. investigated the in vitro and in vivo activity of omipalisib in chemotherapy-sensitive and resistant BL cell culture models. They exposed BL cells to omipalisib for 24-72 hours and observed significant induction of apoptosis in chemosensitive cells after 72 hours; however, higher concentrations were required to induce apoptosis for treatment-resistant cells. G1 cell cycle arrest occurred 72 hours after omipalisib administration to all cell lines. However, cell cycle arrest occurred at higher concentrations of G2/M in chemotherapy-resistant Raji cells. An in vivo study on mice showed slower tumor progression in mice treated with omipalisib compared to the control group. The present study demonstrated in vivo and in vitro that omipalisib inhibited the PI3K/AKT/mTOR pathway, inducing apoptosis and impairing BL cell proliferation [15]. Bhatti et al. administered the molecule named MK-2206, which inhibits the PI3K/AKT/mTOR pathway, to the BL cell line. They showed in a preclinical study with BL cell lines that MK-2206 was not effective alone, but existing chemotherapeutic agents (doxorubicin +/- dexamethasone combination) increased its efficacy [5]. Another study by Li et al. treated the BL cell lines with NVP-BEZ235, a dual PI3K/mTOR inhibitor. The study revealed the inhibitory effect of NVP-BEZ235 on the proliferation of BL cells, reporting that the anti-apoptotic activity of the agent was dose and time-dependent [16]. There are various studies in the literature on the efficacy of omapalisib for different solid tumors. These studies have shown both its efficacy alone and synergistic effect with combined treatments. It is therefore believed that PI3K/mTOR inhibitors can be a therapeutic option for such cancers [9,17,18,19].

Considering such studies in the literature and the present study, it is suggested that omipalisib and other PI3K/AKT/mTOR inhibitors have antitumoral activities on BL cells with mechanisms such as cytotoxic effect, induction of apoptosis, and cell cycle arrest.

Conclusion

In conclusion, omipalisib has the potential to be a new treatment option, especially for patients with relapsed/refractory BL and intolerance to intensive chemotherapy. Moreover, inhibition of the PI3K/AKT/mTOR pathway is very important in the

treatment of BL since the inhibition of this pathway results in an antitumoral effect. However, the number of studies investigating the effect of omipalisib on BL is limited. Therefore, there is a need for more studies both in vitro and in vivo with omipalisib alone and with combination treatments. Furthermore, more clinical studies are needed to evaluate its efficacy, side-effect profile, effect on survival, and permanent response of patients.

Acknomledgments

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Conflicts of interest

There are no conflicts of interest in this work.

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