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Effect of Bortezomib, Daptomycin and Their Combination on Antiproliferation in U266 Multiple Myeloma Cell Line

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ABSTRACT

Multiple myeloma is the second most common hematological malignancy in adults. Although current treatment approaches extend survival to 6 to 10 years in multiple myeloma treatment, most patients relapse. This situation has led to the need for new therapeutic agents in the treatment of multiple myeloma. Daptomycin, a drug molecule isolated from Streotomyces roseosporus and used especially in infections caused by Gram-positive bacteria, has been shown in recent studies to suppress tumor migration and angiogenesis. Bortezomib is a chemotherapy drug currently used in the treatment of multiple myeloma. In this study, we determined the antiproliferative effect of Bortezomib and Daptomycin applications on the U266 multiple myeloma cell line by % cell viability analysis with the XTT method. In addition, we determined the apoptosis levels of U266 multiple myeloma cell lines by flow cytometry. In conclusion, we determined that the combined application of Bortezomib and Daptomycin increased the anticancer effect of Bortezomib alone in U266 multiple myeloma cell lines. In light of the data obtained from this study, we can say that the effect of Daptomycin added to Bortezomib in the treatment of multiple myeloma may contribute significantly to the treatment of the disease.

Keywords: Multiple Myeloma, Bortezomib, Daptomycin, Proliferation, Apoptosis.

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Introduction

Multiple Myeloma is a type of cancer characterized by clonal proliferation of malignant plasma cells in the bone marrow, accounting for 10% of hematologic malignancies. The most common abnormality in myeloma is abnormalities involving the immunoglobulin heavy chain replacement site (on the long arm of chromosome 14) [1].

There are 20,000 new diagnoses each year in the United States. The median age at diagnosis is 70 years. The primary site of involvement is the bone marrow, and involvement is usually in the form of lytic lesions throughout the skeletal system. In multiple myeloma, factors produced by neoplastic plasma cells lead to bone damage, which is the main feature of the disease. Myeloma cells disrupt normal B lymphocyte function, leaving the body vulnerable to infection. Although immunoglobulin levels are increased due to increased M protein in the plasma, functional antibody production is very low. This leads to suppression of humoral immunity in patients and an increased risk of bacterial infection [2].

Daptomycin is a natural, bactericidal antibiotic produced by a bacterium called Streptomyces roseosporus, which has a cyclic lipopeptide structure of 13 amino acids. This structure causes the formation of transmembrane channels in the lipoteichoic acid found abundantly in the walls of gram-positive bacteria, thus causing intracellular ions (potassium and magnesium) and ATP to leak through these channels. This leads to a rapid depolarization in the membrane potential of gram-positive bacteria. The loss of membrane potential leads to inhibition of protein, DNA, and RNA synthesis, which quickly leads to the death of the bacteria [3]. The reason why opening holes in the membrane to allow the flow of ions during the membrane binding stage does not create toxicity for mammalian cells is that the lipid tail of the drug enters itself and creates channels that allow the flow of ions without tearing the membrane. This explains the mechanism that kills bacteria but does not create toxicity for mammalian cells [4].

Daptomycin exhibits in vitro bactericidal activity against Gram-positive pathogens, including strains with limited therapeutic alternatives. In addition, Daptomycin is an option for the treatment of pediatric infections caused by Vancomycin-resistant enterococci (VRE) such as urinary tract infection, bacteremia, sepsis, and endocarditis [5,6]. Daptomycin has weak in vitro activity against Gram-negative bacteria. Daptomycin is used as a broad-spectrum antibiotic in patients with dominant immunity [7].

Bortezomib is one of the most effective chemotherapy drugs used in the treatment of multiple myeloma. Bortezomib, a proteasome inhibitor, is widely used. In the event of recurrence of cancer, its use contributes to the treatment of the disease. Bortezomib has apoptosis-inducing effects by disrupting the cell cycle in cells. It is well tolerated in patients after use. Therefore, its use can be continued in outpatient treatment. Bortezomib is

known to have synergistic effects with some drugs. Its use together with compounds such as dexamethasone increases the efficiency of the drug with a synergistic effect. Therefore, there is a need to investigate new molecules that will synergistically increase the effect of Bortezomib [8].

In a study, it was reported that Daptomycin can bind to human ribosomal S19 protein (RPS19) and suppress tumor migration and angiogenesis. In this study, MCF7, HepG2, Huh7, SK-BR-3, MDA-MB231, A549, HeLa, HCT116, CCD-18Co, HUVECs and HEK293 cell cultures were used and it was stated that the culture with the highest sensitivity was the breast cancer cell line MCF7. Their findings show that anti-cancer drugs that will target RPS19 may be promising [9].

In this study, in order to determine whether Daptomycin has an effect on the anticancer effect of Bortezomib, which is used as a chemotherapy drug, we examined its anti-proliferative effect on U266 multiple myeloma cell lines using the XTT method and its effect on apoptosis using the Annexin V/Dead Cell kit using a flow cytometry device.

Materials and methods

Cell Culture

Multiple Myeloma (U266) cells obtained from ATCC were grown in RPMI (1:1) cell culture medium containing 1% L-glutamine, 1% penicillin-streptomycin and 10% fetal bovine serum in 25 cm2 flasks under sterile conditions at 37°C and 5% CO2 [10].

XTT Cell Viability Test

The effect of bortezomib and daptomycin on U266 cell viability was investigated with the XTT (2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) test in accordance with the kit protocol [11].

Evaluation of Apoptosis with Flow Cytometry

Apoptosis was evaluated with the Muse Annexin V/Dead Cell (Merck Millipore, Darmstadt, Germany) test. The procedure to be applied was determined according to the manufacturer's instructions [12].

Statistical Analysis

Statistical evaluation of the data to be obtained and drawing of the graphics were done using the Grahped Prism program.

Results and Discussion

XTT assay in Multiple Myeloma U266 cell lines

The effects of Bortezomib doses of 1600 nM, 800 nM and 400 nM and Daptomycin doses of 10 mM, 5 mM, 2.5 mM and 1.25 mM and synergistic doses of these two drugs applied to U266 Multiple Myeloma cell lines on % cell viability were investigated using the XTT method (Figures 1-3.).

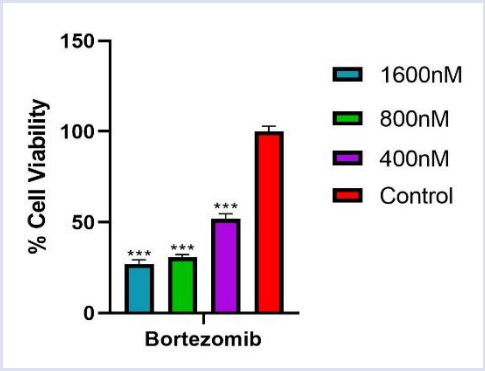

Figure 1. Viability percentages of U266 cells after 24 h Bortezomib exposure (***P<0.001, **P<0.01, *P< 0.05). IC50 392,4

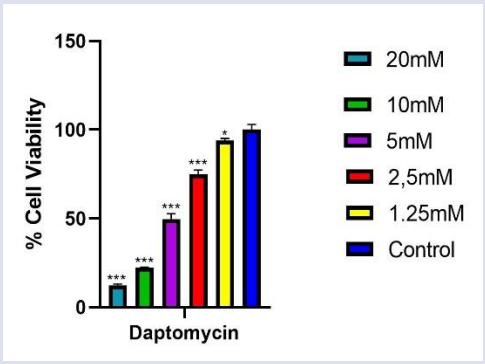

Figure 2. Viability percentages of U266 cells after 24 h Daptomycin exposure (***P<0.001, **P<0.01, *P<0.05). IC50 4,97

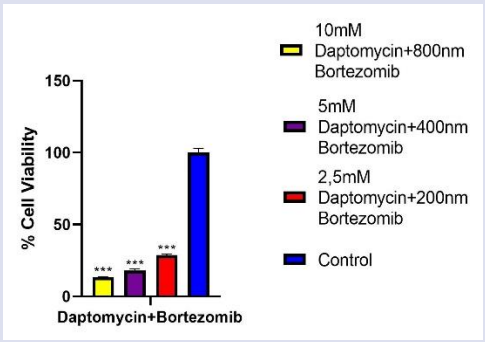

Figure 3. Viability percentages of U266 cells after 24 h Daptomycin+Bortezomib exposure (***P<0.001, **P<0.01, *P<0.05).

The IC50 values of Bortezomib and Daptomycin applied to U266 cell lines for 24 hours were determined as 392.40 nM and 4.97 mM, respectively. It was determined that Daptomycin and Bortezomib, applied separately and together to U266 Multiple Myeloma cancer cell lines for 24 hours, produced an anticancer effect on cancer cells. It was also observed that the combined application of Daptomycin and Bortezomib caused a significant decrease in cell proliferation.

Flow Cytometry Analysis in Multiple Myeloma U266 Cell Lines

The effects of Bortezomib and Daptomycin alone and together on apoptosis in U266 Multiple Myeloma cell lines were investigated by flow cytometry (Figure 4.).

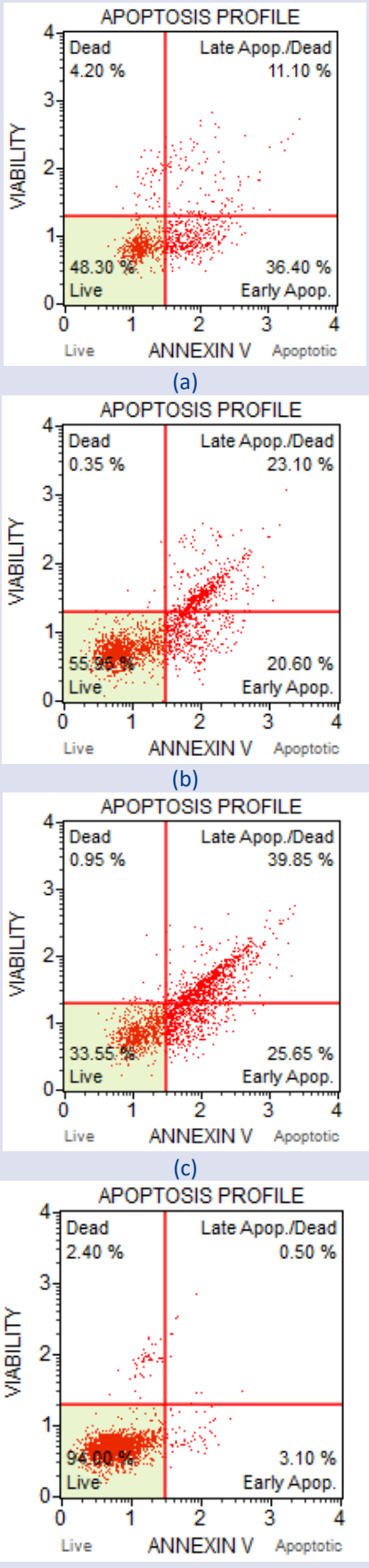

Figure 4. Flow cytometry graphs (A) IC50 dose of Bortezomib applied (B) IC50 dose of Daptomycin applied (C) IC50 doses of Bortezomib and Daptomycin applied (D) Control group U266 cell lines

Early and late apoptosis levels in U266 cell lines treated with IC50 doses of Bortezomib and Daptomycin were found to be increased compared to control group U266 cell lines. In addition, early and late apoptosis levels in U266 cell lines treated with IC50 doses of Bortezomib + Daptomycin were found to be significantly reduced compared to single drug application.

Conclusions

In our study, we determined that even low doses of Bortezomib applied to U266 Multiple Myeloma cancer cell lines had high anticancer effects. In addition, we determined that Daptomycin, an antibiotic drug, also had anticancer effects on U266 Multiple Myeloma cancer cell lines. Interestingly, we showed that the combined use of Bortezomib and Daptomycin on U266 Multiple Myeloma cancer cell lines increased their anticancer effects.

Bortezomib, a chemotherapy drug also known as PS341, has inhibitory properties of the 26S proteasome found in cell nuclei and cytosol. This inhibition is reversible. The 26S proteasome is found in eukaryotic cells and functions in the degradation of protein molecules responsible for the continuation of the cell cycle [13,14]. Abnormal proteins are formed especially as a result of mutations that occur due to the rapid and uncontrolled division of cells, especially in Multiple Myeloma. However, there may be deformations in the folding of the synthesized proteins. The 26S proteasome mediates the elimination of these proteins This situation mediates a decrease in the proliferation of cancer cells and constitutes the main mechanism of anti-cancer effects [15]. The ubiquitin-proteasome pathway is a pathway that regulates the biological processes of cells [16]. It is also important that bortezomib is the first drug to be approved by the US Food and Drug Administration (FDA) as a drug that inhibits this ubiquitin-proteasome pathway. The use of bortezomib mediated the direction of cells to apoptosis by suppressing the expression of proteins such as BCL-2 and BCL-XL, which are apoptosis inhibitors, in in vitro cancer cell lines. In addition, it has shown anticancer activity by regulating the expression of proteins involved in DNA repair [17]. Although bortezomib is a powerful chemotherapeutic, as with all chemotherapy drugs, resistance can develop in cancer cells against this drug [18]. However, unwanted side effects such as neuropathy may occur in its use in hematological cancers [19].

Bortezomib has anticancer effects in cancer cell lines other than Multiple Myeloma. In a study investigating its effect on drug-resistant human neuroblastoma cell lines, it was determined that apoptosis was induced in both drug-resistant and drug-resistant neuroblastoma cell lines and that it inhibited the cell cycle even at nanomolar concentrations, thus having a high anticancer activity. However, it was determined that these cell lines reduced cell proliferation after Bortezomib application after being taken into the in vivo environment. In addition, it was determined that Bortezomib inhibited new vessel formation in xenograft models [20]. It was shown that

Bortezomib was applied alone and in combination to the A549 cancer line, which is one of the non-small cell lung cancer cell lines, causing a pause in the cell cycle and reducing cell proliferation [21]. In the study examining the in vitro and in vivo effect of Bortezomib on prostate cancer, it was determined that proteasome inhibition and prostate-specific antigen modulation increased positively when the Bortezomib dose given to the patients was increased. Based on this, it has been reported that the combination of Bortezomib with radiotherapy and chemotherapy in the treatment of prostate cancer may have a higher effect on the increase of anticancer effect [22]. The use of Bortezomib also has effects on osteoblast cells that play a role in bone formation. Surprisingly, unlike other cancer cells, Bortezomib mediates an increase in the number of osteoblast cells. This also causes an increase in bone tissue [23]. The results of our study are parallel to the data in the literature that apoptosis is induced and proliferation is reduced in cancer cell lines treated with Bortezomib in vitro conditions.

Daptomycin is an antibiotic with a cyclic lipopeptide structure. It is produced by Streptomyces roseosporus. Its production is possible by adding decanoic acid to the growth medium of the medium. Daptomycin is used for the treatment of infections occurring in the skin and skin structures. It is also an antibiotic approved by the FDA [24,25]. Daptomycin is an effective drug used against Gram-positive bacteria, as well as in the treatment of sepsis, meningitis and many diseases such as endocarditis [5]. However, the increase in Daptomycin use causes bacteria to develop resistance to this antibiotic. It is known that Staphylococcus aureus in particular develops resistance to this drug [26,27].

There are very few literature studies on the relationship between Daptomycin and cancer. In a study on Daptomycin and cancer, it was shown that the binding of Daptomycin to ribosomal protein S19 has anticancer effects. It has been reported that the complex of Daptomycin and ribosomal protein S19 mediates a decrease in cell proliferation without inducing apoptosis in cell lines. When ribosomal protein S19 expression was reduced using siRNA, it was determined that the anticancer efficacy of Daptomycin application in MCF-7 cell lines increased. Daptomycin also suppressed the formation of new vessels and migration of cancer cells by suppressing VEGF expression in cancer cell lines. The data obtained from this study suggested that Daptomycin may be an alternative tool in cancer treatment [9]. In our study, we determined that Daptomycin, whose anticancer effect was reported, had a effect on the chemotherapeutic effect of Bortezomib, which is currently used in the treatment of Multiple Myeloma. We determined that Bortezomib and Daptomycin had anticancer effects on U266 cancer cell lines when used alone. However, we have shown that the combined use of Bortezomib and Daptomycin can create a great effect on U266 cell lines, making a significant contribution to the efficiency of Multiple Myeloma treatment.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgement

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