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Investigation of The Effect of B-108 Containing Azomethine Group on DNA Repair Gene

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Research Article

History

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ABSTRACT

Objective: Cancer arises as a result of the failure of the mechanisms controlling normal division in a group of cells. It is known that some new synthesis compounds intended for use in cancer treatment have anti-fungal, anti-bacterial, anti-carcinogenic effects. In this study, it was aimed to apply the newly synthesized B-108 compound to the A-549 cell line and then to investigate the effect of this compound on the *ERCC1* gene expression profile.

Materials and Methods: Firstly, compound B-108 was synthesized in our study. Afterwards, this synthesized molecule was applied in eight different concentrations (1-100 µg/ml) in A-549 cell line and 3-(4,5-dimethylthiazol-2-yl)-2,5-yl for 24 hours, 48 hours and 72 hours. Anticancer activities were determined using diphenyltetrazolium bromide (MTT) method. Expression level of DNA repair gene (*ERCC1*) was determined using RT-PCR method.

Results: As a result, it was determined that the molecule applied to the A-549 cell line showed the highest activity after 72 hours of incubation. It was observed that the *ERCC1* gene expression of the molecule applied on lung cancer was lower than the control group.

Discussion: Considering the current study results, low expression of *ERCC1* shows that compound B-108 correlates with overall survival on lung cancer cells.

Keywords: Lung cancer, Azomethine Group, DNA Repair, Gene Expression

Azometin Grubu İçeren B-108' in DNA Tamir Geni Üzerine Etkisinin Araştırılması

Süreç

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ÖZ

Amaç: Kanser, bir grup hücrede normal bölünmeyi kontrol eden mekanizmaların çalışmamasının bir sonucu olarak ortaya çıkmaktadır. Kanser tedavisinde kullanılması amaçlanan bazı yeni sentez bileşiklerin anti-fungal, anti-bakteriyel, anti-kanserojenik etkileri olduğu bilinmektedir. Bu çalışmada yeni sentez B-108 bileşiğinin A-549 hücre hattına uygulanması ve daha sonra bu bileşiğin *ERCC1* gen ekspresyon profili üzerine etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmamızda ilk olarak B-108 bileşiği sentezlenmiştir. Daha sonra bu sentezlenen molekül A-549 hücre hattında sekiz farklı konsantrasyonda (1-100 µg/ml) uygulandı ve 24 saat, 48 saat ve 72 saat süreyle 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromid (MTT) yöntemi kullanılarak antikanser aktiviteleri saptandı. DNA tamir geninin (*ERCC1*) ekspresyon düzeyi RT-PCR yöntemi kullanılarak belirlendi.

Sonuçlar: Sonuç olarak A-549 hücre hattına uygulanan molekülün 72 saatlik inkübasyon sonrasında en yüksek aktiviteyi gösterdiği tespit edildi. Akciğer kanseri üzerine uygulanan molekülün *ERCC1* gen ekspresyonunun kontrol grubuna göre daha düşük olduğu gözlendi.

Tartışma: Mevcut çalışma sonuçları değerlendirildiğinde, *ERCC1* düşük ekspresyonu B-108 bileşiğinin akciğer kanser hücreleri üzerinde genel sağkalım ile korele olduğunu göstermektedir.

Anahtar sözcükler: Akciğer Kanseri, Azomethine Group, DNA Tamiri, Gen Ekspresyonu

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Introduction

Lung cancer is the most frequently diagnosed cancer in the last decade, and its incidence is high due to poor survival and high mortality [1]. Histologically, lung cancer is divided into two categories as small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). While chemotherapy and chest radiotherapy containing cisplatin are applied to patients with limited-stage SCLC, combinations of drugs such as etoposide, cisplatin, and paclitaxel are applied to patients with extensive-stage SCLC. These treatment modalities increase symptom relief and survival rates [2]. NSCLC carcinoma accounts for the majority of lung cancer, with approximately 40% being adenocarcinoma, 25%-30% squamous cell carcinoma, and 10%-15% large cell carcinoma [3]. Among the current chemotherapy agents used in the treatment of NSCLC, there are targeted drugs such as docetaxel, cisplatin, etoposide, paclitaxel, gemcitabine, and vinorelbine, and these drugs increased 1-year survival rates by 35% and 2-year survival rates by 15%. This treatment approach generally leads to reduction of symptoms and prolongation of life expectancy [4]. Recent advances in the diagnosis and treatment of cancer have been observed with the development of targeted drugs. In addition, the benefits from chemotherapy differ among patients, resulting in response or resistance to treatment [5] While platinum-based chemotherapy provides benefits in the metastatic setting, it also contributes to adjuvant therapy as a supplement. However, the survival rate in this treatment strategy is 10–12 months [6]. Platinum-based drugs suppress tumorigenesis and support lung cancer treatment by making intra-chain and inter-chain cross-links in tumor tissues [7]. Platinum-based drugs are among the standard chemotherapy methods in NSCLC patients [8]. Azomethine groups are an organic ligand formed by the condensation of ketones or aldehydes with a primary amino compound and coordinating metal ions via -N=CH- nitrogen [9]. (Table 1). *ERCC1* is a gene associated with resistance to platinum-based chemotherapy [10]. ERCC1 is an endonuclease that cuts the damaged segment of DNA and has a rate-limiting role in the nucleotide excision repair-mediated repair of platinum inserts [11, 12]. Cisplatin is an important mechanism in DNA repair. Blocking of cells by cisplatin causes the target drug to bind to DNA and to reveal platinum-DNA adducts. These additives inhibit DNA replication by forming cross-covalent bonds between DNA strands. Mismatch excision repair has been associated with resistance to platinum-based chemoreception . In vitro studies have associated *ERCC1* gene expression with platinum resistance in cell lines in testicular, ovarian, bladder, and NSCLC cancers [13]. High expression of *ERCC1* has been reported to have a higher survival rate compared to low expression in patients with recently resected NSCLC, resulting in better outcomes by reducing tumor recurrence of the repair system [14].

Materials and Methods

Synthesis of 9,9'-(((2-hydroxypropane-1,3-diyl) bis (oxy))bis(2,1 phenylene))bis (met-hanylylidene)) bis (azanylylidene)) bis (ethane-2,1-diyl))bis (8,9,10, 11, 20,21-hexa-hydro-7H,19H-dibenzo [f,q][1,5] dioxa[9,12,15] triazacyclooctadecin-20-ol) (compound B-108)

0.37 mmol of dialdehyde **1** was dissolved in 5 ml of hot acetonitrile. Subsequently, 1.37 mmol of tris(2-aminoethyl) amine was added to the reaction mixture, which vigorously stirred for 3 hours (Figure **1**). At the end of reaction time, the reaction solution was poured into an ice-water mixture and left for 20 minutes. Afterwards, the solution was vigorously stirred with the addition of sodium chloride and the yellowish precipitate is formed, which was filtered, washed with distilled water and dried. Yield 31%, m.p.167-168°C. ^{1}H NMR spectrum: (DMSO-d_6, δ, ppm), 2.44 m (4H, 4CH), 2.68-2.8 m (22H, 11CH_2), 3.47 m (18H, 9CH_2), 4.11-4.20 m (30H, 15CH_2), 6.85-7.14 m (24H, 24CArH), 7.28-7.42 m (12H, 12CArH), 7.71-7.86 m (12H, 12CArH), 8.50-8.61 m (12H, 12CH=N). ^{13}C NMR spectrum: (DMSO-d6, δ, ppm), 61.89 (CH_2), 62.67 (CH_2), 67.95 (CH_2), 68.05 (CH_2), 73.09 (CH_2), 73.13 (CH), 73.14 (CH), 115.61 (C_{Ar}H), 115.62 (C_{Ar}H), 115.69 (C_{Ar}H), 115.72 (C_{Ar}), 122.05 (C_{Ar}H), 126.01 (C_{Ar}), 126.02 (C_{Ar}H), 127.23 (C_{Ar}), 127.41 (C_{Ar}H), 132.42 (C_{Ar}), 157.09 (CH=N), 157.71 (CH=N). MALDI-TOF MS: 2170. Found, %: C 72.51; H 6.61; N 11.35. $C_{44}H_{52}N_4O_8$. Calculated, %: C 72.41; H 6.68; N 11.26 (Figure 1).

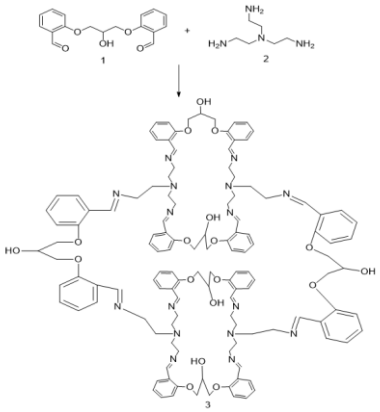

Figure1. The synthesis of macroheterocycle 3

Cell culture

The lung A-549 cell line was incubated in an oven medium in 100 Units/ml penicillin, 10% fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM). When the cells reached 80% growth rate, they were passaged and the medium changed.

In vitro cytotoxicity determination (MTT)

2 ml of Trypsin/EDTA was added to the A-549 cell, which reached a certain growth rate in the flask, and incubated in an oven containing 5% CO_2, allowing the cells to be separated from the flux surface. Growing A-549 cells were passaged into 96-well plates with a cell density of 1 x 10^5 and at the end of 24 hours different concentrations of drugs (1-100 µM) were dosed into

the wells. Anticancer activity of A-549 cell line was determined by the MTT method. After 24, 48 and 72 hours, 10 µL of MTT was added to each well. and incubated for three hours MTT was removed from the well and added to each well. 100µL of DMSO was added and incubated on the mixer for 15 minutes. Then, absorbance values at 570 nm wavelength were read. Measurements were made in 3 replicates for each A-549 cell line and IC50 values were determined using GraphPad Prism7.

Cell Morphology

A-549 cells ($5x10^5$ cells/well) were plated. 1µM of B-108 compound was added to each cell in the wells. Changes in cell morphology were observed using a 20X magnification cell imaging device (ZEISS Axio Vert.A1) and compared with each other.

Isolation of RNA from Cell Culture Samples

IC_{50} concentrations were taken as reference by the values in the MTT method at the end of 48 hours. A-549 cell line grown in fluxes was passaged into 6 wells and drug dosing was done at the end of 24 hours. RNA isolation was performed using the RNeasy Plus Mini kit protocol.

cDNA Synthesis

Appropriate kit protocol was applied to synthesize cDNA from RNA and the synthesized cDNAs were used to determine the expression levels of the *ERCC1* gene by RT-PCR.

Real-Time PCR Analysis

Expression levels of the *ERCC1* gene were analyzed in an RT-PCR device using the optimized RT² SYBRGreen qPCR Mastermix kit. SYBRGreen was used as a fluorescent dye in this study. 25 µl of qPCR mix was prepared from the cDNA-containing samples with reference to the kit procedure. Statistical analysis of the data was done by ΔΔCT method using https://dataanalysis2.qiagen.com/pcr software.

Results

Synthesis of Heterocyclic Compounds Containing Azomethine Group

The targeted chemicals were synthesized in a non-catalytic medium. NMR spectroscopy was used to determine their structure. As it can be seen from the spectra, the signals from the aldehyde and amine groups are missing. Instead, the imine (azomethine) group's signals are seen. As a consequence, we may deduce that macrocycles occur during the process.

In vitro assay for cytotoxicity activity (MTT assay)

B-108 drug in the range of 1-100µM was applied to the A-549 cell line at 8 different concentrations. This molecule was incubated for 24, 48 and 72 hours and analyzed for cell viability by MTT assay. The cytotoxic activities of drug B-108 were determined by comparing the groups with and without drug added. When the findings were evaluated, the highest activity in the B-108 molecule was detected at the end of 72 hours (Figure 2).

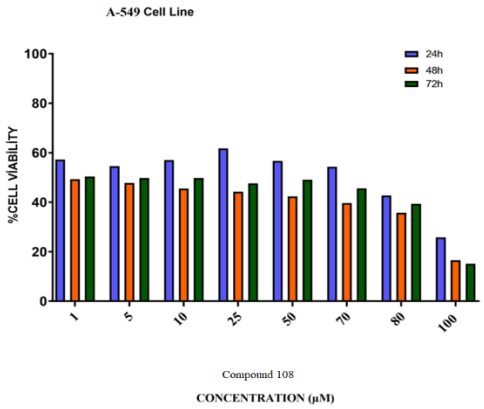

Figure 2. Cytotoxicity study of B-108 in A-549 cells. A-549 cells were treated with this grup for 24, 48, and 72 hours in a concentration range of 1 to 100µM. Represents the mean±SEM of the experiment.

Cell Morphology Analysis

Morphological analyzes in cell line A-549 were performed after 72 hours of incubation of 10µM compound B-108. It was determined that there was a significant change in the morphology of the A-549 cell line in which the B-108 compound was added compared to the control group in the A-549 cell line (Figure 3).

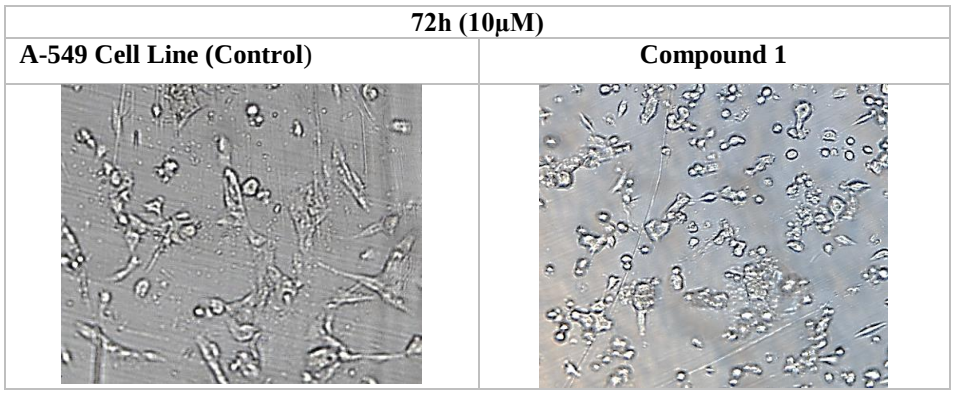

Figure 3. Morphological changes of A-549 cells after 72 hour of incubation with concentration (10 µM) of B-108 the result presented are from that were carried out and photographed microscopically

Gene Expression Analysis

The expression level of the *ERCC1* gene used in the study is shown in the figure 4. *ERCC1* gene expression level of B-108 molecule applied on lung cancer was found to be lower than the control group ($p<0.000$) (Figure 4).

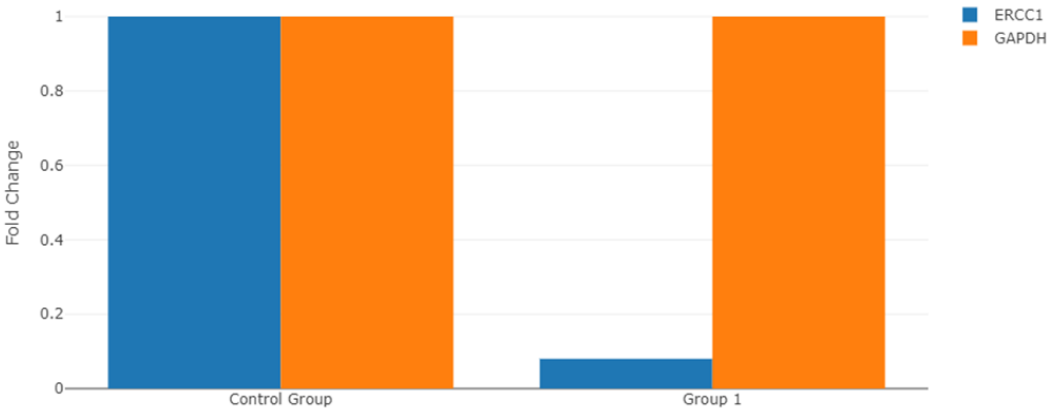

Figure 4. Comparison of the expression level of *ERCC1* gene in the group.

Discussion

When the results of the study were examined, it was determined that the B-108 molecule applied on the A-549 cell line showed the highest activity at the end of 72 hours. It has been determined that the anticancer activity of the B-108 molecule on the cancer cell is high. Azomethine groups is an organic ligand formed by the condensation of ketones or aldehydes with a primary amino compound and coordinating metal ions via -N=CH- nitrogen. The use of metal-drug interactions as chemotherapeutic agents is important as they affect DNA cleavage [9]. Therefore, molecules containing azomethine group exhibited anticancer activity [15]. The effects of B-108 molecule with anticancer activity on *ERCC1* gene expression levels in A-549 lung cancer cells were investigated. Expression of genes involved in DNA repair in lung cancer cells is associated with chemotherapy resistance. Among these genes, the most preferred and studied is *ERCC1* [16]. ERCC1 is an enzyme that repairs DNA damage caused by the action of platinum [17]. Low expression of this gene causes an increased incidence of lung cancer, while high expression is associated with cisplatin resistance by causing rapid repair of damaged DNA in cells paused in the G2/M phase during the cell cycle [18]. In a study, it was suggested that high expression of *ERCC1* is associated with platinum resistance and poor prognosis [17]. In some studies, it has been determined that cisplatin-based chemotherapy increases the survival rate in patients with low *ERCC1* expression [19]. Dong et al. shows that patients with low *ERCC1* expression have a higher 5-year survival rate than patients with high expression on *ERCC1* expression and the prognosis of platinum chemotherapy in patients with advanced NSCLC [20]. In a study by NSCLC, it was seen that the low expression level of *ERCC1* in patients with NSCLC also showed a good prognosis in platinum-based chemotherapy treatment [21]. In the relationship of

ERCC1 to immunotherapy response, it has been understood that the decrease in ERCC1 level increases errors in tumor cell DNA, resulting in stronger immune response. Chabanon et al. demonstrated that the low expression level of *ERCC1* against increased neo-antigens in tumor cells resulted in a better response to immunotherapy [22]. In our study, it was determined that the *ERCC1* gene expression level of B-108 drug was lower in A-549 cells compared to the control group. In conclusion, low expression of *ERCC1* shows that compound B-108 correlates with overall survival on lung cancer cells.

Conclusion

According to the result of this study, B-108 drug may be promising on lung cancer. This study is the first in vitro study and it is important because it is the first study to apply these drugs to A-549 cells for the first time and to determine gene expression levels. Based on the findings of this study, further studies are planned with active targeting of drug B-108.

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