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Research Article

Determination of apoptotic effects of newly synthesized avobenzone Sm(III) complex in non-small cell lung cancer

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ABSTRACT

Cancer is a worldwide prevalent disease characterized by the uncontrolled division and proliferation of cells in an organ or tissue. The most common cause of cancer-related deaths is lung cancer. In this study, we aimed to investigate the apoptotic effects of the lanthanide complex TBK-23, which was used in biological imaging due to its magnetic and spectroscopic properties. The study utilized HTB-54 and BEAS-2B cell lines. The cells were subjected to TBK-23 and cisplatin at active doses for a duration of 24 hours. Subsequently, flow cytometry was employed to assess the quantity of apoptotic cells, while quantitative RT-PCR was used to measure the expression levels of BAX, BCL-2, and BCL-xL. While cisplatin at 31 µM increased the proapoptotic BAX expression level in HTB-54 and BEAS-2B cells, TBK-23 increased BAX in cancer cells at the same concentration, but there was no observable effect in healthy cells. TBK-23 was found to have higher inhibitory effect on the expression of the antiapoptotic BCL-2 gene in cancer cells compared to that of cisplatin-treated cells. Consequently, it was determined that TBK-23, a novel metal-based compound, exhibited apoptotic effects similar to that of cisplatin and could be a potential chemotherapeutic agent.

Keywords: Lung cancer, apoptosis, chemotherapy, lanthanide complex.

1. INTRODUCTION

According to the World Cancer Research Fund International 2020 estimates for lung cancer in the world. There is information on 2,206,771 patients who have been diagnosed with lung cancer worldwide. Of these, 41,264 were from Turkey, and 37,070 of them ended in fatalities.[1] Cancer is a worldwide prevalent disease characterized by the uncontrolled division and proliferation of cells in an organ or tissue. [2] The origin of cancer formation involves environmental and genetic factors such as exposure to carcinogens and replication errors in DNA repair mechanisms [3]. Lung cancer is the most commonly diagnosed cancer type worldwide after women breast cancer and ranks as one of the leading causes of cancer-related deaths. [4] Lung cancer is primarily divided into two main types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is the most common type of lung cancer, accounting for approximately 80-85% of all cases.[5] Although recent advancements in the treatment of lung cancer have been promising, there is still a significant need for the identification and development of more effective therapeutic agents. [6]

Platinum compounds such as cisplatin, oxaliplatin, and carboplatin are well-known metal-based chemotherapeutic anti-cancer drugs.[7] Due to the rapid development of resistance by cells to this group of drugs and their associated adverse effects, there is a need for

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the synthesis of new metal complexes with low toxicity. These complexes should not only exhibit beneficial effects but also overcome the issues of drug resistance and minimize adverse side effects.[8]

Lanthanide complexes have been widely used for imaging purposes in biological systems due to their spectroscopic and magnetic properties. They exhibit unique luminescent properties, making them valuable tools for various imaging techniques such as fluorescence imaging and magnetic resonance imaging (MRI) in biomedical research and diagnostics. In recent years, lanthanide complexes have gained attention not only for their use in cellular DNA targeting but also for their potential as anticancer drugs due to their unique properties.[9] Previous studies have reported that lanthanide complexes possess significant biological activities such as DNA binding and antioxidant activity. These findings highlight the potential of lanthanide complexes as therapeutic agents in the field of cancer treatment. [10]

In our previous study, we investigated the cytotoxic properties of newly synthesized lanthanide complexes on lung cancer cells. We found that the TBK-23 lanthanide complex exhibited low-dose cytotoxic effects specifically on lung cancer cells while having no impact on the same dose on healthy cell lines.[11] This suggests that TBK-23 complex holds potential as a selective cytotoxic agent for lung cancer treatment. Based on this significant finding, the aim of our study was to determine the anticancer activities of the TBK-23 lanthanide complex on lung cancer cells through apoptosis. By focusing on the apoptotic pathways, we aimed to investigate the mechanism of action of TBK-23 complex and its potential as an effective treatment for lung cancer.

2. MATERIAL AND METHODS

2.1. Cells and culture conditions

In this study, lung epidermoid carcinoma (HTB-54) and lung bronchial epithelial normal cell lines (BEAS-2B) were used. Cells were cultured in RPMI-1640 (Gibco, USA) cell medium containing 10% fetal calf serum (FBS; Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in an incubator at 37 °C and 5 % CO_2.

2.2. Preparation of chemicals

In our previous study, the effect of TBK-23 lanthanide complex on cell viability was shown in HTB-54 cells [8.] According to the results of our previous study, the IC_{50} value of the TBK-23 complex was determined as 31 µM. Therefore, in this study, the TBK-23 complex was prepared by dissolving it in dimethyl sulfoxide (DMSO) at concentrations of 31 µM and a low dose of 15 µM. Cisplatin (Sigma, USA), commonly used as a positive control, was used, while the DMSO ratio in the highest concentration was used as the negative control.

2.3. Fluorescence microscopy experiments

To determine the fluorescent properties of TBK-23, HTB-54 lung cancer cells were treated with an effective dose of TBK-23. After overnight incubation, cells were removed with the help of Trypsin-EDTA and placed on slide. Fluorescence imaging was performed at 20X magnification with the aid of an Olympus BX53 fluorescent microscope with U-LH100-HG mercury lamp attachment.

2.4. Apoptosis induction assay

The cells detached using trypsin-EDTA and seeded into six-well cell culture plates to achieve a concentration of $1x10^6$ cells/ml. Different concentrations (15 and 31 µM) of TBK-23 lanthanide complex and cisplatin were exposed to 70-80 % confluent cells for 24 hours. The next day, following removal of the cells with trypsin-EDTA, cells were washed with PBS and the apoptotic cell population was determined using the Annexin V-FITC Apoptosis Staining/Detection Kit from Abcam, United Kingdom and analyzed using the BD Accuri C6 Plus Flow Cytometer (BD, USA) according to the recommendations of the manufacturer.

2.5 Determination of the apoptotic gene expression by quantitative real-time PCR

"Different concentrations (15 and 31 µM) of the TBK-23 lanthanide complex and cisplatin were exposed to cells seeded to six-well culture plates at a concentration of $1x10^6$ cells/ml in a CO2 incubator for 24 hours. Following incubations cells were removed by using lysis buffer containing β-mercaptoethanol. RNA isolations were performed using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. Subsequently, single-stranded cDNA was synthesized from the RNA samples using the RT2 First Strand Kit (Qiagen, Germany). Gene expression analysis of apoptosis-associated genes (BAX, BCL-2 ve BCL-xL) were determined by using gene-specific primer sets and SYBR Green Kit (Table 1). Reactions were held in Rotor Gene Q real-time PCR instrument with the following thermal conditions: 15 minutes at 95 °C, 40 cycles of 15 sec at 95 °C, 30 sec at 60 °C, 30 sec at 72 °C, followed by melting curve analysis between 60-95 °C. As an internal control GAPDH was used in Real-Time PCR experiments.

2.6. Statistical Analysis

GraphPad Prism 8 software (San Diego, CA) was used for the statistical analysis of the data. Student's t-test was performed for pairwise comparisons, and analysis of

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variance (ANOVA) was conducted for multiple comparisons. The $2^{-\Delta Ct}$ method was used for the calculation of gene expression levels. A p-value less than 0.05 was considered statistically significant for all results.

Table 1. Gene-specific PCR primer pairs used in the study and their properties.

Oligo name	Sequence (5'- 3')
BAX-F	GTCGCCCTTTTCTACTTTGCC
BAX-R	TGGTCACGGTCCAACCACC
BCL2-F	ATAACGGAGGCTGGGATGC
BCL2-R	TCACTTGTGGCCCAGATAGG
BCL-xL-F	TCAGCCACCATTGCTACCAG
BCL-xL-R	CCAAGGAGCTGGTTTAGGGG
GAPDH-F	GATCATCAGCAATGCCTCCT
GAPDH-R	TGTGGTCATGAGTCCTTCCA

3. RESULTS AND DISCUSSION

In order to determine the fluorescent properties of TBK-23, HTB-54 lung cancer cells were treated with an effective dose of TBK-23 and transferred on a slide for fluorescence microscopy (Figure 1).

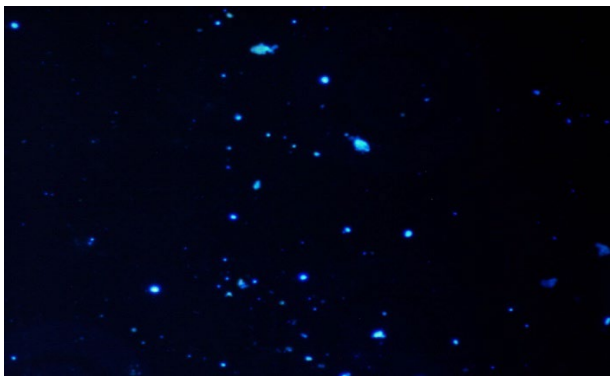
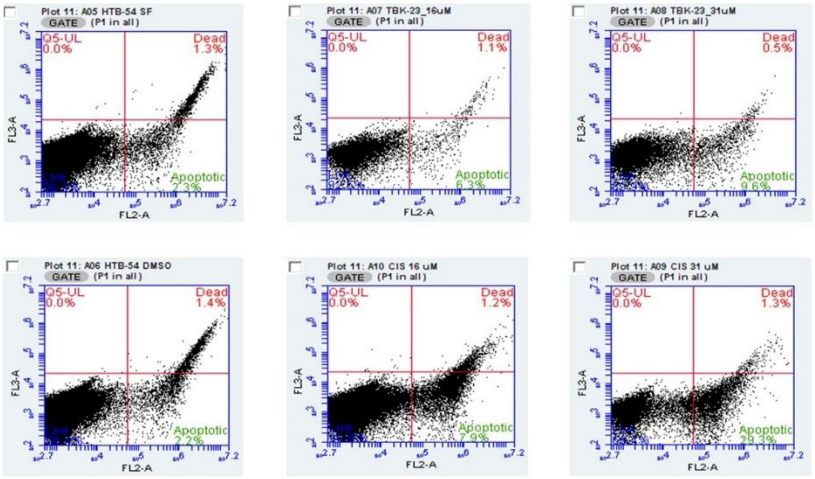


Figure 1. Visualization of HTB-54 lung cancer cells treated with TBK-23 under a fluorescent microscope.



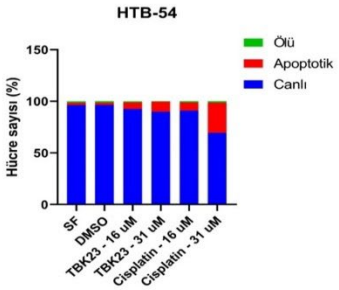
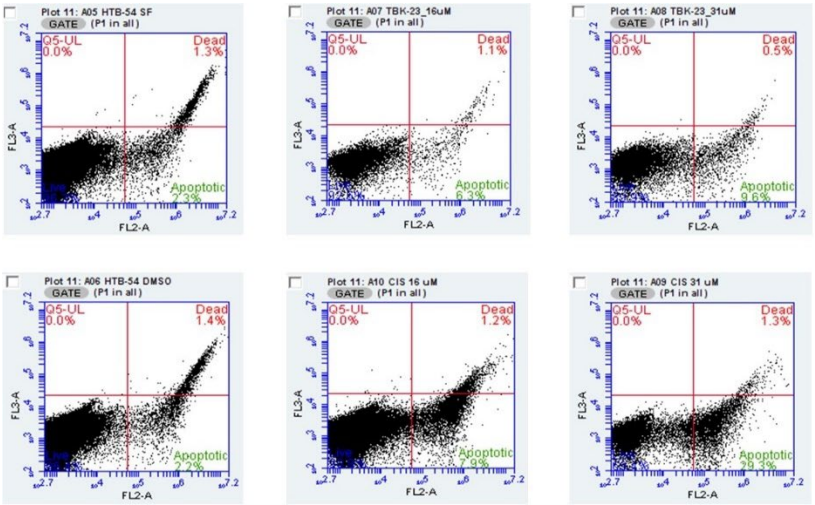


Figure 2. Effect of TBK-23 and cisplatin on apoptosis in HTB-54 lung cancer cells.



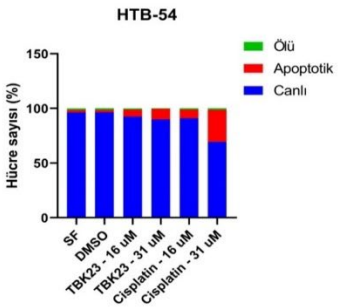


Figure 3. Effect of TBK-23 and cisplatin on apoptosis in BEAS-2B cells.

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Moreover, apoptosis experiments were performed to evaluate the effect of TBK-23 lanthanide complex on cell death in lung cancer cell. After exposure to 16 µM TBK-23 in HTB-54 lung cancer cells, the apoptotic cell population was 6.3%, while it was 9.6% at 31 µM concentration. After cisplatin application at the same concentrations, apoptotic cell populations were 7.9% for 16 µM and 29.3% for 31 µM (Figure 2). In contrast, in BEAS-2B cells, which are healthy cells, after 16 µM and 31 µM TBK-23 exposure, the rate of apoptotic cells was found to be 3% and 3.4%, respectively. Similarly, the rate of apoptotic cells was 3% after 16 µM cisplatin administration, and 4.4% after 31 µM cisplatin administration (Figure 3). Next, RNA was isolated from cells exposed to different concentrations of TBK-23, and expression levels of apoptosis-related genes were analyzed by Real-Time PCR. Expression levels of pro-apoptotic BAX, anti-apoptotic BCL2 and BCL-xL genes were determined. It was observed that the expression level of BAX gene increased significantly compared to the DMSO group as a result of 16 µM and 31 µM cisplatin exposure in BEAS-2B cells, but TBK-23 did not have any significant effect on the BAX gene. In addition, exposure to 31 µM TBK-23 was found to significantly reduce the expression of the BCL2 and BCL-xL genes compared to cisplatin (Figure 4). In HTB-54 lung cancer cells (16 µM and 31 µM), the expression level of the BAX gene increased significantly after exposure to TBK-23 and cisplatin, and the 31 µM concentration of the TBK-23 complex substantially decreased the expression level of the BCL2 gene compared to the cisplatin and DMSO group (Figure 4).

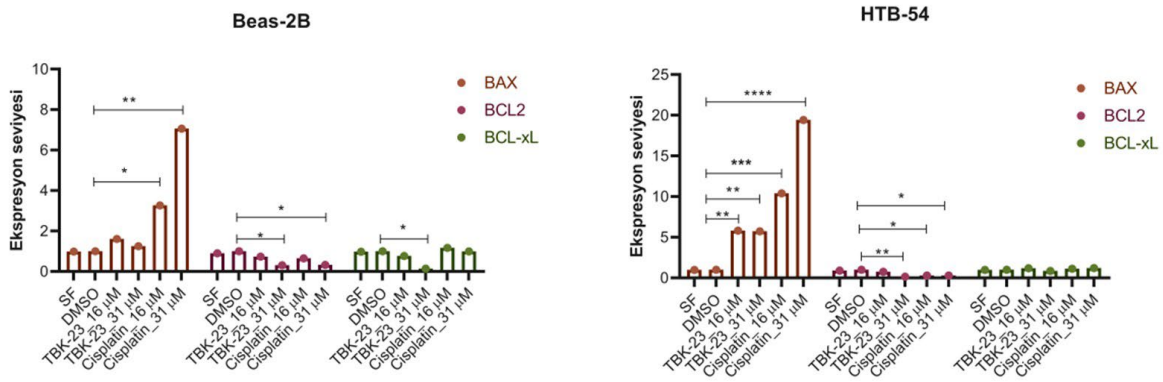

Figure 4. The expression level of apoptosis-related genes is shown in HTB-54 and BEAS-2B cells after administration of TBK-23 and Cisplatin at different concentrations. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

NSCLC is among the deadliest cancers with a mortality rate of more than 50% within one year of diagnosis.[12] In addition to surgical interventions, NSCLC also has a variety of treatment options, including radiation, immunotherapy, and chemotherapy. In most cases, a combination of some or all of these treatments is necessary to help eliminate the cancer. Cisplatin is a widely used chemotherapeutic agent in NSCLC and is usually administered concurrently with radiotherapy. Cisplatin works by interfering with the DNA replication process in rapidly dividing cancer cells. When administered, cisplatin enters the cancer cells and forms covalent bonds with the DNA molecules, specifically binding to the purine bases. This binding prevents the DNA strands from separating properly during replication, leading to the disruption of DNA synthesis and repair mechanisms. Cisplatin poses challenges due to its limited ability to distinguish between healthy human cells and cancerous cells, resulting in significant side effects that include nephrotoxicity, peripheral neuropathy, and severe nausea and vomiting [13]. Therefore, new metal-based chemotherapeutic agents are needed to improve the quality of life of patients and increase survival rates. Metal-based lanthanide complexes, when considering their significant biological activities such as DNA binding and antioxidant effects, hold promising potential for cancer treatment.[14]

The B-cell lymphoma (BCL) protein family has crucial roles in the regulation of cell death mechanisms, apoptosis, necrosis, and autophagy. Under normal conditions, proapoptotic members such as BAX are inhibited by anti-apoptotic factors such as BCL-xl and BCL-2 so that healthy cells do not undergo apoptosis. However, in situations where stimuli such as DNA damage or the formation of large protein aggregates arise, such as in the case of cancer, the expression levels of certain genes may become irregular and lose control [15]. Hence, the overexpression of these proteins in various types of tumors leads to neoplastic growth by inhibiting the BAX protein, thereby preventing cancer cells from undergoing apoptosis. Furthermore, the increased expression of these anti-apoptotic genes is strongly associated with a poor prognosis of cancer patients [16].
In our study, we have found that TBK-23, a lanthanide complex, has a high cytotoxic effect at low doses while demonstrating reduced toxicity on healthy cells. This suggests that TBK-23 could potentially serve as a new metal-based agent, similar to cisplatin, with apoptotic effects that may be equal to or even more effective than

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cisplatin. We attempted to demonstrate the effects of the TBK-23 complex on apoptosis using flow cytometry and RT-PCR methods. Our results indicate that TBK-23 significantly triggers cellular apoptosis, while not exerting a significant effect on healthy cells. In support of our findings, gene expression experiments revealed an increase in pro-apoptotic BAX activity, along with a significant decrease in the expression levels of anti-apoptotic genes BCL-xl and BCL-2 in lung cancer cells treated with TBK-23. These results further validate our observations. In conclusion, our findings demonstrate significant anti-cancer effects of TBK-23 lanthanide complex, suggesting its potential as a chemotherapeutic agent. Further extensive studies are crucial to explore the anti-cancer properties of TBK-23 lanthanide complex in more depth.

Ethical Approval

This study did not involve the use of any human or animal tissues.

Financial Support

This study did not receive any financial support.

Conflict of interests

I declare that there is no a conflict of interest with any person, institute, company, etc.

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