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Research Article

IN VITRO COMPARISON OF THYMOQUINONE AND CISPLATIN EFFECTS ON LUNG CANCER CELL LINES

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ABSTRACT

In our study, we planned to examine the effects of chemical substances on lung cancer cell lines. It is stated in the literature that cisplatin is effective in the treatment of lung cancer. Therefore, in this study, the effects of thymoquinone and cisplatin on lung cancer cell lines were investigated. Lung cancer diagnosis is late. Without symptoms, a nodule in the lung may grow or spread out of the lung. Therefore, 80% of these patients cannot be operated. This situation led to the need to study different molecules in the investigation of nonsurgical treatments. In our study, lung cancer cell lines; A549 (bronchioloalveolar carcinoma), HTB54 (epidermoid carcinoma lung), BEAS2B (bronchus epithelium),that reproduced with passaging by cell culture, were exposed to thymoquinone (tq) and cisplatin (cis) and the effects of different molecules on cancer cells were investigated. A549, HTB54, and BEAS2B were reproduced in culture medium and thymoquinone and cisplatin were administered at 10, 100, and 200 µM concentrations. Live cell determination was determined by MTT (3- (4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide). After MTT was evaluated at 550 nm with a spectrophotometer. The effects of 100 and 200 µM doses of thymokinone in A549, HTB54 and BEAS2B cells were higher than cisplatin effect at the same doses. Cisplatin has no effect at a dose of 100 µM in HTB54, and A549 cells. Both chemicals are effective at 100 and 200 µM doses in BEAS2B cells. The determined effective dose of thymokinone was 100 µM and cisplatin was 200 µM. Both chemicals have similar toxicity at effective doses. As a result, the effect of thymokinone on lung cancer cells was superior to the effect of cisplatin.

INTRODUCTION

Due to the anatomical structure of the lung, the diagnosis is usually late in lung cancer. Without symptoms, the pulmonary nodules can grow and spread out of the lung. Therefore, the disease can be detected in the spreading period. Lung cancer is the leading cause of cancer-related death worldwide(1). The decision to treat lung cancer is mainly based on tumor histology, stage of disease and age, pulmonary function and associated diseases , and specific characteristics associated with the patient, such as concomitant diseases. The 5-year survival rate for non-small cell lung cancer (NSCLC) is 18% , survival is 60-80% for patients stages 1 and 2, and undergoing anatomic resection (2). Localized stage 1 and stage 2 disease accounts for 30% of all NSCLC. The standard treatment approach for non-metastatic stage 1 and 2 patients who are medically suitable for surgery without mediastinal invasion is surgery (2,3). As a general approach, the surgical procedure is applied in cases predicted for R0 resection and selected cases for R1 resection. Adjuvant chemotherapy (CT) is indicated in all patients with resected pathological stage 2 and in stage 1B patients with a size of 4 cm and above(4). In adjuvant chemotherapy, platinum-based chemotherapies are recommended and the most studied KT protocol is vinorelbine + cisplatin combination (5). Stage 3 disease is a highly heterogeneous group, which has been controversial in many aspects of treatment. According to the eighth staging system, hilar tumors with a size greater than 5 cm, intrapulmonary and peribronchial lymph node involvement (T3N1) or tumors greater than 7 cm without lymph node involvement (T4) are in this group. There was no difference in the clinical N descriptor in the seventh and eighth staging. A new definition of T3 / T4 N3 disease was defined as stage 3C (6,7). If no mediastinal lymph node is detected in the pathological staging of locally advanced stage disease and R0 resection is predicted, treatment is the resection of the primary tumor (8). Surgical metastasectomy is the most common treatment option for patients with oligometastatic NSCLC. Surgical treatment has been accepted especially in metastases of the contralateral lung, brain, and adrenal glands. Aggressive local treatment in metastatic and primary areas is recommended for patients classified as M1b stage. Systemic chemotherapy should be

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applied after surgical treatment in multiple metastatic patients (2-5). Long-term disease control is possible with treatment of primary tumor and matastasis (9). Cisplatin-containing combinations are generally preferred in patients receiving chemotherapy. Therefore, thymokinone, the bioactive component of Nigella sativa, was compared with cisplatin at the same doses. Although targeted therapies have a essential place in stage 4 disease, they have no place in stage 3 disease treatment (10).Treatment of patients with stage 4 NSCLC; disease prevalence, number and site of metastasis, presence of symptoms associated with metastasis, histological type, molecular analysis. Approximately 20% of all lung cancers are neuroendocrine carcinoma, 14% of them are small cell lung cancer (3). Small cell lung cancer has the feature of fast doubling time, high growth fraction, and early metastasis. Therefore, the surgical approach is only possible in 2% to 5% of cases (11). The anticancerogenic, antitumoral, and immune-enhancing effects of thymokinone which is the Bioactive component of Nigella Sativa (Black Cumin) have been shown in conducted studies (12,13). In our study, lung cancer cell lines (A549, HTB54, BEAS2B) were passivated in cell culture and exposed to thymokinon (tq) and cisplatin (cis) and their effects were investigated. Cell viability was evaluated and the effects were compared in respective doses. It is thought that if there is a positive effect on the available types of cancer, it can be used as a local or systemic medication in the later stages after phase studies.

MATERIEL & METHOD

A549 cell lines (ATCC LGC Promochem, Teddington, UK) are epithelial carcinoma cell lines in 5% fetal bovine serum-containing RPMI-1640 cell medium, HTB-54 cell lines (ATCC, LGC Promochem, Teddington, UK) are lung epidermoid carcinoma cell lines containing 5% fetal bovine serum in RPMI-1640 cell wells andBEAS-2B cell lines (ATCC, LGC Promochem, Teddington, UK) are bronchial epithelial cell lines transformed with simianvirus 40, will be reproduced bovinpituiter extract containing keratinocyte medium. For the cells, culture medium consisting of 500 mL DMEM, 50 mL FCS and 5 mL Penicilin streptomycin mixture was used. The cells to be used for the experiments were grown in a 75 cm2 culture vessel (Falcon; BD Biosciences, USA) which contains an incubator at 37 ° C, 5% CO 2 and 95% humid environment. Cells were washed with 5 mL of HBSS (Hank Solution Balanced Salt Solution). Cells were liberated by breaking the ligaments with 1X trypsin-EDTA exposure. The trypsin effect was neutralized with culture medium. The cells in the culture dish were transferred to a 50 mL sterile tube and centrifuged at 1500 rpm for 4 min. After centrifugation, the supernetant was aspirated and 5 mL of cells culture medium was added to the cell.For cell counting, 20 µL of cell suspension, 20 µL of 0.5% trypan-Blue and 160 µL of cell medium mixture were prepared. The mixture was transferred to the Thoma lamina (Marienfeld, Germany) and the living cells in the slide were counted. According to the results ,cells diluted with DMEM with 10% FCS were plated in 24 cell culture dishes. The cells were incubated for about 24 hours until 70% confluency. The chemicals to be used in the study were weighed 8 mg in a sensitive scale and taken into 1 mL sterile tubes. The compound in the tube was diluted with DMSO to 500 µM and homogenization was achieved by vortexing. The concentrations (10,100,200 µM) to be used in the assay were prepared separately for each compound. As the negative control, sterile DMSO was used to dissolve the high concentration. After 24 hours, DMEM was aspirated from the cells to 70% confluency and negative control and chemicals were added to the concentrations prepared in the wells. Total fluid volume was completed to 500 µL. After the procedure was completed, the cells were allowed to incubate for 24 hours. As a positive control, cis was used for chemotherapy treatment in the market. Live cell count was determined by a staining method based on the reduction of 3- (4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) by mitochondrial enzymes. By taking 100 µM from the wells with color change, ELISA reader was taken into cell culture (96 well) containers. The changing colors were evaluated at 550 nm with a spectrophotometer. All experimental protocols were repeated at least three times in cell viability experiments. The data obtained from the studies were analyzed for normal distribution and one-way analysis of variance (ANOVA) for normal distribution and Kruskal Wallis test for abnormal distribution. A549 cell line was taken for experiments at 14. Passage HTB54 cell line at 4. Passage and BEAS2B cell line at 12. Passage.

RESULTS

The effects of tq on 100 µM and 200 µM concentrations were statistically significant when compared with DMSO in A549 cells ($p<0.001$). However, in the experiment, tq were found to be toxic at a concentration of 200 µM on A549 cells . When A549 cells are compared with DMSO, the effects of cis at 200 µM concentration are statistically significant, but the effect in 100 µM concentration is not statistically significant ($p<0.001$). The effect of tq at 100 µM concentration on A549 cells is statistically more significant than the effect of cis at a concentration of 100 µM($p<0.01$-100 µM Cis), the effect of tq on the concentration of 200 µM is statistically more significant than the effect of cis at the concentration of 200 µM($p<0.01$-200 µM Cis) (figure 1)

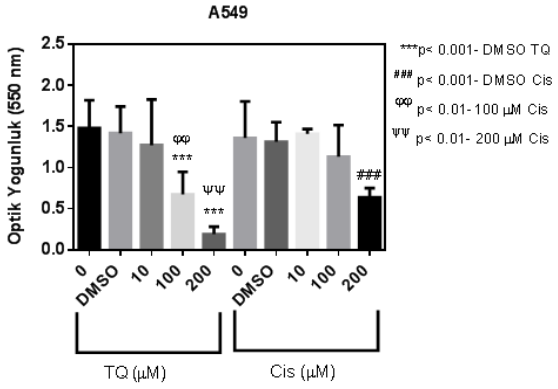

Figure 1 Statistical analysis of Tq and Cis in A549 cells

Effects of tq on 100 µM and 200 µM concentrations were statistically significant in BEAS-2B cells compared to DMSO ($p<0.001$). BEAS-2B cells were evaluated as statistically significant at 100 and 200 µM concentrations compared to DMSO ($p<0.001$). The effect of tq at 100 µM concentration on BEAS-2B cells is statistically more significant than the effect of cis at 100 µM concentration ($p<0.001$-100 µM Cis), the effect of tq at the concentration of 200 µM is statistically more significant than the effect of cis at the concentration of 200 µM ($p<0.001$-200 µM Cis). It is statistically seen that tq is more lethal in BEAS-2B normal bronchial epithelial cells than cis at the same concentrations. In this respect, cis is considered superior to tq. However, it has a similar effect in effective doses (100 for Tq, 200 for Cis) (figure 2)

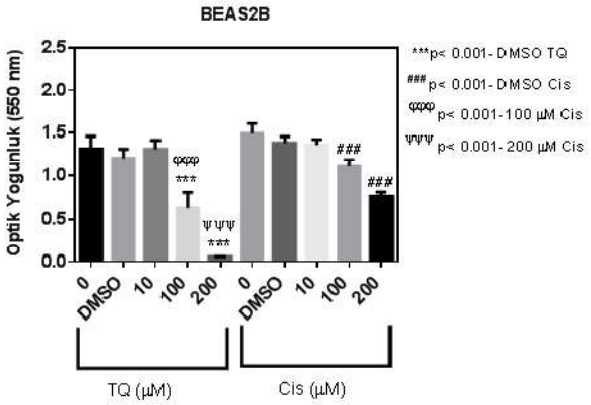

Figure 2 Statistical analysis of Tq and Cis in BEAS-2B cells

When HTB-54 cells were compared with DMSO, the effects of tq with 100 µM and 200 µM concentrations were statistically significant ($p<0.001$). However, the experiment showed that tq was toxic at a concentration of 200 µM on HTB-54 cells. When HTB-54 cells are compared with DMSO, the effects of cis at a concentration of 200 µM are statistically significant, but the effect in 100 µM concentration is not statistically significant ($p<0.001$). The effect of tq at 100 µM concentration on HTB-54 cells is statistically more significant than the effect of cis at 100 µM concentration ($p<0.001$-100 µM Cis), the effect of tq at the concentration of 200 µM is statistically more significant than the effect of cis at the concentration of 200 µM ($p<0.001$-200 µM Cis). (figure 3)

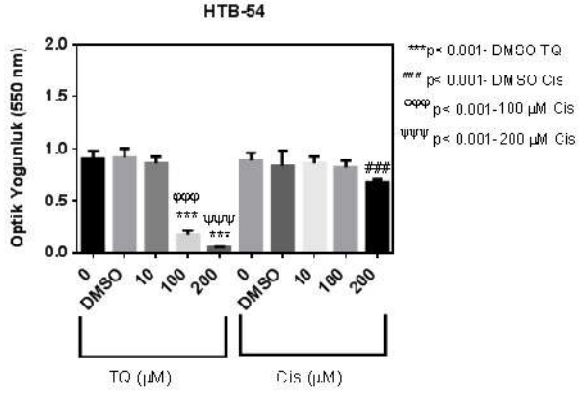

Figure 3 Statistical analysis of Tq and Cis in HTB-54 cells

DISCUSSION

The 5-year survival rate for non-small cell lung cancer (NSCLC) is 18%, for stages 1 and 2, and for patients undergoing anatomic resection, survival is 60-80% (2). The standard treatment approach is surgery in non-metastatic stage 1 and 2 patients who are medically fit for surgery and have no mediastinal invasion(2,3). Stage 3 is a highly heterogeneous group of patients, with many aspects of treatment being controversial. If the mediastinal lymph node is not detected in the pathological staging of the local advanced stage disease and if R0 resection is prescribed, the treatment is the resection of the primary tumor (8). Chemotherapy is the treatment of choice in local advanced disease, which is decided by the multidisciplinary approach in which R0 resection cannot be performed after induction chemotherapy or diagnostic procedures. Usually combinations containing cisplatin are preferred. Therefore, in our study, thioquinone, a bioactive component of Nigella sativa, was compared with cisplatin at the same doses.

Nigella Sativa (black cumin); it is known as fertile grain ,black seed, black cumin among the people in our country, and are frequently used. In our study, the bioactive component tq from the essential oil of N.sativa was used. Many previous studies have shown that antifungal, anti-bacterial, antiinflammatory, analgesic, antioxidant, hypoglycemic, immune-system-enhancing effect, as well as antitumoral and anticancerogenic effects of black cumin (12,13).

In A549 cells, the effects of tq with 100 and 200 µM and the concentration of cis with 200µM are statistically significant. The effect of tq at 100 µM and 200 µM concentration is statistically more significant than the effect of cis. In invitro conditions in lung bronchoalveolar carcinoma, tq has been shown to be superior to cis. The effect of 100 µM and 200 µM tq in HTB-54 cells at 200 µM concentration is statistically significant. The effect of cis at 100 µM concentration is not statistically significant. Therefore, the effect of tq at 100 µM concentration is statistically more significant than the effect of cis at both 100 µM and 200 µM concentration. In invitro conditions in lung epidermoid carcinoma, tq has been shown to be superior to cis.

In BEAS-2B bronchial epithelial cells, the effects of tq and cis at 100 µM and 200 µM concentrations were statistically significant. The effective dose of tq in cancer cells is 100 µM concentration. Hence, both substances have effective doses (100 µM for tq and 200 µM for cis), it appears to have about 50% lethal effect on BEAS-2B normal bronchial epithelium.

In our study, the effect of tq on lung bronchoalveolar carcinoma and epidermoid carcinoma cells in invitro conditions was shown to be statistically more significant than the effect of cis. In normal bronchial epithelial cells, the same concentration was statistically more effective at tq in invitro conditions, whereas at effective doses (100 µM for tq and 200 µM for cis), it was seen that they had a similar effect on normal bronchial epithelium. Therefore, tq is superior to cis at effective doses. After the necessary studies in lung cancer, it is thought to use tq as a treatment option.

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Authors' contributions

Our study is a specialty thesis in medicine within the scope of the project. Contribution has been provided by all authors at every stage of the article.

Availability of data and materials

All data obtained in our study are record and available.

Ethics approval and consent to participate

The study was carried out on cancer cell lines in the cell culture laboratory and was carried out in accordance with international standards.

Competing interests

All authors declared that there is no conflict of interest.

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