Mesothelioma and Small cell lung cancer; effects of Nigella Sativa thymoquinone on cell lines

ABSTRACT

Background: Lung cancer and malignant mesothelioma are types of cancer with a poor prognosis and fatal. Small cell lung cancer is much more aggressive and survival shorter than non-small cell lung carcinoma. Mesothelioma is a rare malignant disease that commonly affects the pleura. Cisplatin is frequently used in chemotherapy protocols. Thymoquinone is a chemical with antineoplastic effects procured from the Nigella Sativa plant. It was aimed to investigate the effects of thymoquinone and cisplatin on small cell lung cancer and mesothelioma cell lines.
Methodology: The study was done in the Cell Culture Laboratory of Gaziantep University. Cell lines of small cell lung cancer, malignant pleural mesothelioma and non-cancerous bronchial epithelium were used in the study. Cells were cultured in dimethyl sulfoxide. The effective doses of thymoquinone and cisplatin were calculated. Accordingly, which were detected doses of thymoquinone as 100 µM and cisplatin as 200 µM. The viability of cells were evaluated using 3-(4,5-dimethylthiazol-2-il) 2,5-diphenyl tetrazolium bromide test. Experiments were repeated 4 times at different times by the same team in the same laboratory. Statistical analysis of the study was done using the Chi-square test. The study was was accordance with international standards on cell lines in the laboratory.
Results: Chemical treats were administering on all cell lines at doses of 100 µM and 200 µM. Thymoquinone at a dose of 100 mm; viability of cells were detected in 48% in mesothelioma, 44% in small cell lung cancer and 55% in noncancerous epithelium cell lines. Cisplatin at a dose of 200 µM; viability of cells were detected in 63% in mesothelioma, 48% in small cell lung cancer and 59% in noncancerous epithelium cell lines. There was no significant toxicity of dimethyl sulfoxide used as a chemical solvent when compared with physiological saline.
Conclusions: Thymoquinone at a dose of 100 µM was more effective than cisplatin at a dose of 200 µM on both small cell lung cancer and malignant pleural mesothelioma cell lines. Cisplatin was more effective in small cell lung cancer than malignant pleural mesothelioma at a dose of 200 µM. The effects of thymoquinone were similar in both cancer cell lines.

Key words: cell lines, cisplatin, lung cancer, mesothelioma, thymoquinone

INTRODUCTION

Lung cancer and malignant mesothelioma are types of cancer with a poor prognosis and fatal. Lung cancers are categorized as small cell (SCLC) and non-small cell lung carcinoma (NSCLC). SCLC is 14% of all lung cancers. SCLC is much more aggressive and survival shorter than NSCLC. 60% of SCLC is a stage 4 metastatic tumor at the time of diagnosis [1,2]. Unfortunately, surgical treatment cannot be done in lung cancers with the distant metastasis. Chemotherapy is used in lung cancers at this stage. Cisplatin is among the combinations in these chemotherapy protocols [3]. Mesothelioma, which occurs due to asbestos exposure, is a rare malignant disease that commonly affects the pleura [4,5]. Thymoquinone is a bioactive chemical procured from the Nigella Sativa plant. Its shown to have antitumoral and antineoplastic effects [6]. Therefore, it was aimed to investigate the effects of thymoquinone and cisplatin on SCLC and mesothelioma cell lines.

MATERİALS AND METHOD

The study was done in the Cell Culture Laboratory of Gaziantep University. American Type Culture Collection (ATCC) cell lines were maintained in accordance with international standards in the laboratory. Cell lines of small cell lung cancer (CRL-5853 ATCC-NCI-H1048), malignant pleural mesothelioma (CRL-5820 ATCC-NCI-H28), non-cancerous bronchial epithelium (BEAS-2B ATCC) and non-cancerous pleural epithelium were used in the study (Figure 1A). Cell lines were cultured in dimethyl sulfoxide solution (DMEM) with 10% fetal bovine serum (FBC; Gibco, USA) and 1% antibiotic (Gibco, USA), at 37°C and 5% CO2 in medium of Roswell Park Memorial Institute (RPMI). Available in the laboratory, thymoquinone (2-Isopropyl-5-methyl-1,4-benzoquinone) produced in accordance with international standards was used in the study. The effective doses (ED50) of thymoquinone and cisplatin were calculated by administering different doses on the cultured cell lines. Accordingly, the ED50 of thymoquinone as 100 µM and the ED50 of cisplatin were determined as 200 µM. Cells were cultured in 10% FBS (RPMI-appropriate medium) for 24 h in 96-well plates (containing 2500/ml cells). The medium was replaced with serum-free medium (for 16 hours) prior to chemical exposure. Cells were treated separately with 100 µM thymoquinone and 200 µM cisplatin for 4 hours under incubation conditions (Figure 1B). DMEM, the solvent of chemical substances, was used as negative control group. The cell viability was evaluated using 3-(4,5-dimethylthiazol-2-il) 2,5-diphenyl tetrazolium bromide test (MTT) in accordance with international standards. These procedures were done on all cell lines. Experiments were repeated 4 times at different times by the same team in the same laboratory. Statistical analysis of the study was done using the Chi-square test. The study was was accordance with international standards on cell lines in the laboratory.

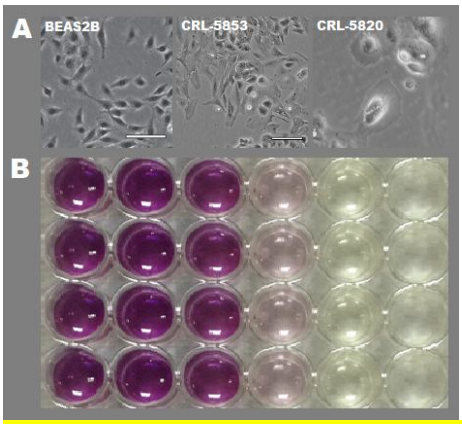

Figure 1. Images of cell lines and chemically treated cells
A.Image of cell lines (ATCC) B. Image of cells incubated after chemical treated

RESULTS

The chemical solvent DMSO was used as the negative control group. The toxicity of the solvent was compared with that of physiological saline (SF) in the study. ED50 of thymoquinone as 100 µM and the ED50 of cisplatin were determined as 200 µM in the preliminary study. Chemical treatments were administering on all cell lines at doses of 10,100,200 µM. Cell death by SF administering wasn't detected in any of the cell lines. The toxicity of DMSO wasn'nt observed on any tumoral or nontumoral cell lines. Cell lines of non-cancerous pleural epithelium lysis in RPMI due to temperature changes during incubation and could not be used. Therefore, BEAS2B (the bronchial epithelium) was used as the nontumoral cells.

The effect of thymoquinone at 10 µM doses on malignant pleural mesothelioma (CRL-5820) was statistically significant$^{(p<0.003)}$. However, there was no significant effect of cisplatin when compared with DMSO at this doses. 100 µM thymoquinone$^{(p<0.001)}$ was more effective on malignant mesothelioma cell lines than both the same and 200 µM doses cisplatin. At 200 µM doses, both chemicals were effective $^{(p<0.001)}$. Thymoquinone was more effective than cisplatin at 200 µM doses$^{(p<0.001)}$. However, these doses were lethal effect for thymoquinone and effective dose for cisplatin (Figure 2).

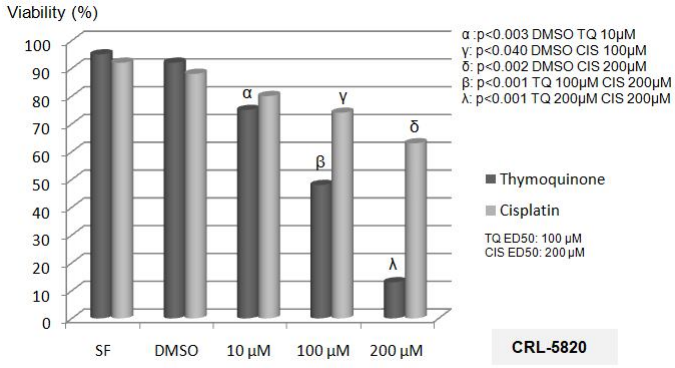

Figure 2. Viability of cells after chemical exposure in pleural malignant mesothelioma

There was no statistically significant effect of either chemical at 10 μM doses in small cell lung cancer cell lines (CRL-5853). Thymoquinone 100 μM doses were more effective than both 100 μM$^{(p<0.001)}$ and 200 μM$^{(p<0.03)}$ doses of cisplatin. The effects of 100 μM$^{(p<0.05)}$ and 200 μM$^{(p<0.004)}$ cisplatin were statistically significant compared to DMSO. However, the effect of 100 μM cisplatin on cell viability wasn't at least 50%. Thymoquinone was more effective than cisplatin at 200 μM doses$^{(p<0.001)}$. However, these doses were lethal effect for thymoquinone and effective dose for cisplatin (Figure 3).

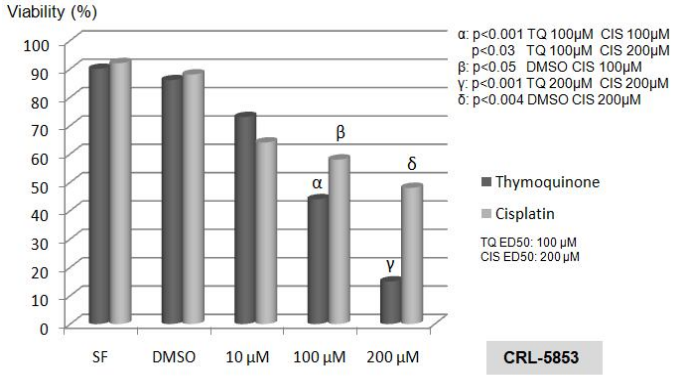

Figure 3. Viability of cells after chemical exposure in small cell lung cancer

There was no statistically significant effect of either chemical at 10 μM doses in non-cancerous bronchial epithelium cell lines (BEAS2B). Thymoquinone 100 μM doses were more effective than 100 μM$^{(p<0.001)}$ doses of cisplatin and, thymoquinone 200 μM doses were more effective than cisplatin at 200 μM doses$^{(p<0.001)}$. There was no statistically significant difference between the effects of both chemicals at effective doses. Therefore, toxicities were similar at effective doses (Figure 4).

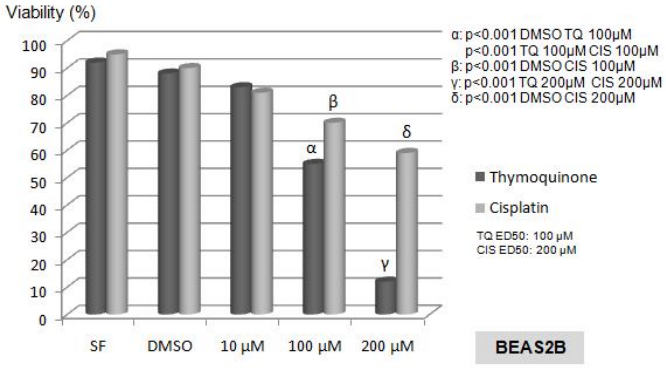

Figure 4. Viability of cells after chemical exposure in non-tumoral bronchial epithelium

DISCUSSION

The lung cancer is the cause of 25% of cancer deaths in both men and women. It is examined in two categories as small cell (SCLC) and non-small cell lung cancer (NSCLC). SCLC comprises about 15% of lung cancers, while NSCLC comprises for 85% [7]. Treatments include surgery, chemotherapy, radiotherapy and immunotherapy. Small cell lung cancer is a progressive disease that can usually be detected at an advanced stage at diagnosis. Therefore, chemotherapy is very important in the treatment of this disease. Despite its cellular toxicity, cisplatin, an alkylating agent, is frequently used [8]. This chemotherapeutic agent is preferred among many combination treatments. Recently, immunotherapy and targeted agents have greatly improved survival in the treatment of NSCLC. Unfortunately, It isn't very successful in SCLC [9]. Platinum is among the chemical agents frequently used in the chemotherapy protocol. Because of, cisplatin was used on the SCLC in the study.

Thymoquinone with a molecular weight of 164.2 g/mol is achieved from the plant Nigella Sative. Studies on the anticancer and antitumoral effects of this bioactive substance reported in the literature. It was proven that thymoquinone significantly inhibited the foci of lung metastases in a the study [15]. It was used to compare thymoquinone with cisplatin because of its antitumoral anticancer activities in our study. Chemicals treated to cell lines had to be dissolved in a solvent. For this purpose, dimethyl sulfoxide solution (DMEM) was used. Both chemicals were dissolved in DMEM. Since each chemical may have toxicity on living cells, a saline (SF) negative control group was made for comparison with DMEM. There was no statistically significant difference between DMEM and saline in all of the mesothelioma, small cell lung cancer and noncancerous bronchial epithelial cell lines. Therefore, it was accepted that the chemical solvent wasn't toxic to living cells. Doses that reduced the viability of cancer cell lines by at least 50% were calculated. This value was specified as the traditional ED50 dose in the study. The ED50 of thymoquinone was 100 µM and that of cisplatin was 200 µM. The effects of both chemicals at ED50 doses were statistically significant when compared with DMSO. At 200 µM doses, thymoquinone was more effective than the cisplatin, but this was unacceptable. Because 200 µM, the ED50 dose for cisplatin, was the lethal dose for thymoquinone. Therefore, the comparison was made at the ED50 doses of the chemicals. Accordingly, 100 µM dose of thymoquinone was more effective than 200 µM dose of cisplatin. No statistically significant difference was found in the comparison of the ED50 of both chemicals on noncancerous bronchial epithelium. Their effects on the bronchial epithelium were found to be similar. As a result, thymoquinone was shown to be more effective than cisplatin in small cell lung cancer cell lines (Figure 3,5).

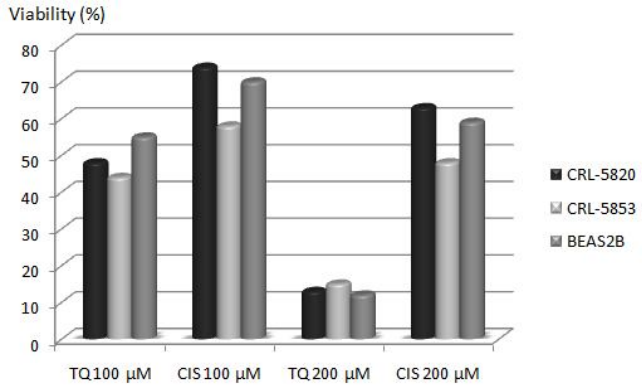

Figure 5. Effects of thymoquinone and cisplatin at effective doses on all cancer cell lines

Malignant mesothelioma is a fatal disease that usually originates from the pleura, rarely from the peritoneum. The main risk factor in malignant mesothelioma is asbestos exposure. Pleural mesothelioma composes for 90% of all mesotheliomas. It has a poor prognosis and a very low 1-year median survival despite chemotherapy [10]. Extrapleural pneumonectomy (EPP) is among the surgical approaches in the treatment of pleural mesothelioma. This method involves resection a lung, parietal pleura, pericardium, and sections of diaphragm. However, a major surgical treatment and it isn't suitable for every patient. Lung parenchyma-sparing surgical approaches have been recommended in recent years [11]. Therefore, pleurectomy decortication are done a common surgical treatment in Malignant pleural mesothelioma (MPM). Hyperthermic intrathoracic chemoperfusion (HITOC) with cisplatin at 42 °C can be performed in the same surgery after completion of pleurectomy decortication [12]. A greater depth of penetration of the chemotherapeutic agent cisplatin administered with HITOC is achieved and induction of signaling pathways for tumor cell apoptosis is activated [13-15]. Because of, cisplatin was used on the MPM in the study.

The effects of both chemicals at ED50 doses were statistically significant when compared with DMSO in MPM. At 200 µM doses, thymoquinone was more effective than the cisplatin, but this value the lethal dose for thymoquinone. Therefore, 100 µM dose of thymoquinone was more effective than 200 µM dose of cisplatin. No statistically significant difference was found in the comparison of both chemicals on noncancerous bronchial epithelium and those were found to be similar. Thymoquinone was shown to be more effective than cisplatin in small cell lung cancer cell lines in malignant pleural mesothelioma (Figure 2,5). The effects of chemicals on cancer cell lines were compared with each other. Although the effect of thymoquinone at a dose of 100 µM was greater in small cell lung cancer cell lines, there was no statistical difference. Thymoquinone was similarly effective in both SCLC and MPM cell lines. Cisplatin was more effective on SCLC cell lines at a dose of 200 µM and it was statistically significant. As a result, cisplatin was more effective than MPM in SCLC cell lines.

CONCLUSIONS

Thymoquinone at a dose of 100 µM was more effective than cisplatin at a dose of 200 µM on both small cell lung cancer and malignant pleural mesothelioma cell lines. Cisplatin was more effective in small cell lung cancer than malignant pleural mesothelioma at a dose of 200 µM. The effects of thymoquinone were similar in both cancer cell lines.

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COMPETING INTERESTS
All authors declared that there is no conflict of interest.

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