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Investigation of The Antiproliferative Effects of Thiocolchicoside on A549 Lung Cancer Cells

Tiyokolşikosid'in A549 Akciğer Kanseri Hücrelerindeki Antiproliferatif Etkilerinin Araştırılması

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ABSTRACT

Objective: This study aims to determine the IC_{50} value of Thiocolchicoside on A549 human non-small cell lung cancer cells using the MTT assay and to investigate its antiproliferative and wound healing effects.

Methods: The cytotoxic and wound healing effects of Thiocolchicoside on A549 cells were evaluated in vitro. Cell viability was assessed at 24, 48, and 72 hours by MTT assay to calculate IC_{50} values. Additionally, an in vitro wound healing (scratch) assay was performed to evaluate cell migration.

Results: The IC_{50} value of Thiocolchicoside at 24 hours was found to be 2.693×10^{-4} M. MTT assay results showed significant cytotoxicity at high concentrations, while lower concentrations had no significant effect or increased cell viability. Cytotoxic effects increased over time, being more evident at 48 and 72 hours. In wound healing assays, low concentrations of Thiocolchicoside significantly enhanced wound closure, whereas higher concentrations reduced this effect. Statistical analysis revealed significant differences between concentrations at 24 and 48 hours, except for the lowest concentration at 24 hours.

Conclusion: Thiocolchicoside exerts dose- and time-dependent cytotoxic effects on A549 cells and may promote wound healing at low concentrations, suggesting potential dual roles in cancer treatment and tissue repair.

Keywords: A549, antiproliferative, thiocolchicoside, wound healing

ÖZ

Amaç: Bu çalışmanın amacı, Tiyokolşikosid'in A549 insan küçük hücreli dışı akciğer kanseri hücreleri üzerindeki IC_{50} değerini MTT testi kullanarak belirlemek ve antiproliferatif ve yara iyileştirici etkilerini araştırmaktır.

Yöntem: Thiocolchicoside'ın A549 hücreleri üzerindeki sitotoksik ve yara iyileştirici etkileri in vitro olarak değerlendirildi. Hücre canlılığı, IC_{50} değerlerini hesaplamak için MTT testi ile 24, 48 ve 72 saatlerde değerlendirildi. Ek olarak, hücre göçünü değerlendirmek için in vitro yara iyileşmesi (scratch) deneyi yapılmıştır

Bulgular: Tiyokolşikosidin 24 saatteki IC_{50} değeri $2,693\times10^{-4}$ M olarak bulunmuştur. MTT test sonuçları yüksek konsantrasyonlarda önemli sitotoksisite gösterirken, daha düşük konsantrasyonların önemli bir etkisi olmamış veya hücre canlılığını artırmıştır. Sitotoksik etkiler zamanla artmış, 48 ve 72. saatlerde daha belirgin hale gelmiştir. Yara iyileşmesi deneylerinde, düşük Tiyokolşikosid konsantrasyonları yara kapanmasını önemli ölçüde arttırırken, yüksek konsantrasyonlar bu etkiyi azaltmıştır. İstatistiksel analiz, 24 saatteki en düşük konsantrasyon hariç, 24 ve 48 saatteki konsantrasyonlar arasında önemli farklılıklar olduğunu ortaya koymuştur.

Sonuç: Tiyokolşikosid, A549 hücreleri üzerinde doza ve zamana bağlı sitotoksik etkiler gösterir ve düşük konsantrasyonlarda yara iyileşmesini destekleyebilir, bu da kanser tedavisi ve doku onarımında potansiyel ikili rollere işaret eder.

Anahtar Kelimeler: A549, antiproliferatif, tiyokolşikosid, yara iyileşmesi

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Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide for both men and women (Siegel et al., 2023). The disease develops in various regions of the bronchial tree, leading to a wide range of symptoms and clinical findings (Lemjabbar-Alaoui et al., 2015). Major risk factors include prolonged tobacco use, as well as air pollution, genetic predisposition, environmental toxins, certain viral infections, and a history of previous pulmonary diseases (Akhtar & Bansal, 2017). The risk of lung cancer increases with age, with the majority of cases occurring in individuals over the age of 65 (Kratzer et al., 2024). Histologically, lung cancer is primarily classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for approximately 85% of all lung cancer cases and is further divided into subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (Belani et al., 2007). These histological differences necessitate diverse treatment approaches. While surgery and adjuvant therapies are emphasized in early stages, chemotherapy, radiotherapy, and targeted therapies are utilized in advanced stages (Wathoni et al., 2022). In newly developed therapeutic strategies, the A549 cell line is frequently used as a model for NSCLC in in vitro experiments.

In recent years, there has been a growing interest in treatment approaches based on natural products, in addition to classical therapies. Innovative methods such as photodynamic and photothermal therapy are being explored, and plant-derived metabolites are increasingly considered as supportive or complementary agents in cancer treatment (Nobili et al., 2009). Alkaloids found in plants represent an important group in this regard, possessing not only anticancer activity but also various pharmacological effects (Olofinsan et al., 2023).

Colchicine, one of these alkaloids, has long been used in the treatment of various inflammatory diseases and has recently gained attention for its anticancer potential. Extracted from *Colchicum autumnale* and *Gloriosa superba*, colchicine disrupts cellular microtubule organization by inducing autophagy in specific cancer cell lines, such as A549 non-small cell lung cancer cells (Bhattacharya et al., 2016; Sivakumar, 2013). Studies have shown that while colchicine exhibits limited cytotoxicity at low doses, its anticancer mechanisms appear promising (Kurek, 2018).

Inspired by the anticancer effects of colchicine, one of its semi-synthetic derivatives, thiocolchicoside, has attracted interest due to its muscle-relaxant properties as well as its potential anticancer activitie (Artusi et al., 2003). Thiocolchicoside inhibits the Nuclear Factor kappa B (NF-κB) signaling pathway, promotes apoptosis, and regulates the p53 protein, making it a promising adjuvant agent (Mahendran et al., 2020; Reuter et al., 2012). Moreover, its selective effects on cancer cells without inhibiting proliferation in healthy cells suggest a favorable toxicity profile (Reuter et al., 2010).

In conclusion, agents derived from natural products constitute a significant area of research aimed at enhancing the efficacy of current treatments and reducing adverse effects in lethal diseases such as lung cancer. Further studies on the anticancer effects of alkaloids like colchicine and its derivative thiocolchicoside may provide novel pharmacological perspectives

Methods

Cell Culture Study

In this study, various cytotoxicity and wound healing assays were performed under in vitro conditions using the A549 human non-small cell lung carcinoma cell line. The A549 cell line was obtained from the stocks of the Department of Pharmacology, Faculty of Pharmacy, Atatürk University, and all experiments were conducted in the cell culture laboratory of the same department. For culturing the cells, a complete medium composed of 89% Essential Minimum Eagle Medium (EMEM), 10% fetal bovine serum (FBS), and 1% penicillin-streptomycin antibiotic (PSA) was used. During the study, routine cell culture procedures—such as thawing, subculturing, passaging, and cryopreservation—were carried out in accordance with standard protocols.

Cells stored in liquid nitrogen in cryovials containing 10% dimethyl sulfoxide (DMSO) were thawed in a 37°C CO_2 incubator. To minimize the toxic effects of DMSO, the cells were immediately diluted with complete medium, centrifuged, and resuspended in fresh medium. The cells were then transferred to 75 cm^2 culture flasks and incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% humidity. The cells were expanded by changing the medium every two days. Once they reached confluency, they were washed with phosphate-buffered saline (PBS), trypsinized, and passaged. After centrifugation, the cells were resuspended in fresh culture medium, transferred into new flasks, and maintained under standard incubation conditions.

To ensure consistency in experimental applications, cell counts were performed prior to each assay. For this purpose, the cell suspension was diluted 1:20 with PBS and trypan blue, loaded onto a Thoma counting chamber, and examined under a microscope to distinguish live (unstained) and dead (blue-stained) cells. Cell numbers were calculated using the formula $N \times DF \times 10^4$ to determine the number of cells per milliliter (N = Number of cells counted, DF = Dilution factor, 10^4 = Conversion factor based on hemocytometer volume).

Thiocolchicoside Application and MTT Cytotoxicity Assay

The MTT assay is based on the principle that the water-soluble compound MTT is reduced by metabolically active cells to an insoluble purple-blue formazan product. Since the dye is water-soluble, its absorbance can be measured spectrophotometrically at specific wavelengths. The amount of formazan formed is directly proportional to the number of viable cells, allowing for the rapid evaluation of multiple samples simultaneously. In this study, the assay was performed using a range of thiocolchicoside concentrations: 1.7×10^{-3} M, 3.2×10^{-4} M, 3.2×10^{-5} M, 3.2×10^{-6} M, and 3.2×10^{-7} M.

Cells were seeded into 96-well plates at a density of 5×10^3 cells per well. After seeding, A549 cells were incubated for 24 hours at 37°C in a 5% CO_2 humidified incubator. Subsequently, various concentrations of thiocolchicoside were applied to the cells, and the cytotoxic effect on cell viability at 24, 48, and 72 hours was evaluated using an MTT assay kit, following the manufacturer's instructions (Acros Organics, China). A 10% MTT solution prepared in sterile PBS was added to each well of 96-well plates. Following 4 hours of incubation at 37°C in a 5% CO_2 environment, 100 µL of DMSO was added to dissolve the formazan crystals.

The absorbance of the resulting formazan was measured at 570 nm using an ELISA reader (Multiskan Sky, Thermo, Singapore). The IC_{50} value was determined using the GraphPad software by applying a nonlinear regression analysis to the log-transformed concentration-response data.

Cell viability in each treatment group was calculated relative to the control group, which was considered 100%, using the formula below. Bar graphs representing cell viability were generated using GraphPad: % Cell Viability = (Mean Absorbance of Sample / Mean Absorbance of Control) × 100

Wound Healing Assay

A549 cells were cultured in T75 cm^2 flasks using EMEM medium supplemented with 10% FBS. After incubation for 24 hours at 37°C with 5% CO_2 and 90% humidity, the cells were trypsinized and seeded into 24-well plates at a density of 2×10^4 cells per well. Upon reaching confluency, a scratch was made in the monolayer of each well using a sterile pipette tip to simulate a wound. Images of the scratch area were captured at 0 hours using an inverted microscope.

Following this, thiocolchicoside was applied at concentrations of 3.2×10^{-4} M, 3.2×10^{-5} M, 3.2×10^{-6} M, and 3.2×10^{-7} M according to IC50 value and the plates were re-incubated under the same conditions. Additional images were taken from the same wound area at 24 and 48 hours. To evaluate cell migration and wound closure, scratch widths at each time point were compared with the baseline measurements. The percentage of wound closure was measured and calculated using ImageJ software.

Wound closure percentages at 24 and 48 hours were calculated using the following formulas:

% Wound Closure (24 hours):

$\%\ \text{Wound Closure}_{(24)} = ((A_0 - A_{24}) / A_0) \times 100$

% Wound Closure (48 hours):

$\%\ \text{Wound Closure}_{(48)} = ((A_{24} - A_{48}) / A_{24}) \times 100$

A_0 = wound area at 0 hours

A_{24} = wound area at 24 hours

A_{48} = wound area at 48 hours (Huang et al., 2023)

Statistical Analysis

The results of MTT assay and the measured wound closure percentages were statistically analyzed using IBM SPSS Version 20. Intergroup comparisons were performed by One-Way ANOVA followed by Post Hoc Tukey test to determine statistically significant differences between groups. Results are presented as mean ± SD. A p-value of less than 0.05 ($p < .05$) was considered statistically significant. Graphs were generated and visually edited using GraphPad Prism version 10.1.1.

Results

Calculation of IC_{50} Value of Thiocolchicoside

The cell viability percentages of each group were used to calculate the IC_{50} value, which is presented in the graph shown in Figure 1. According to the 24-hour MTT analysis, the IC_{50} value of Thiocolchicoside in A549 cells was determined as 2.693×10^{-4} M.

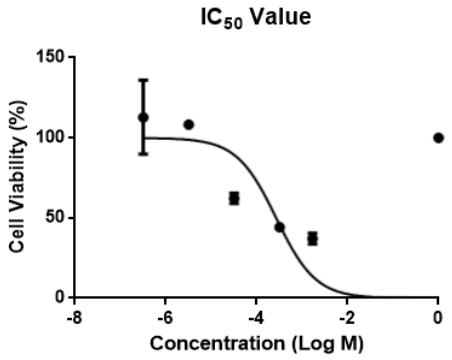

Figure 1.
IC_{50} value of Thiocolchicoside in A549 cells

MTT Cytotoxicity Results

The effects of Thiocolchicoside at concentrations of 1.7×10^{-3} M, 3.2×10^{-4} M, 3.2×10^{-5} M, 3.2×10^{-6} M, and 3.2×10^{-7} M on A549 cell line were evaluated using the MTT assay at 24, 48, and 72 hours alongside the control group. The absorbance value of the control group was accepted as 100% reference.

At 24 hours, as shown in Figure 2, cell viability percentages were found to be 37.16%, 44.29%, and 62.25% for Thiocolchicoside concentrations of 1.7×10^{-3} M, 3.2×10^{-4} M, and 3.2×10^{-5} M, respectively. These results demonstrated a statistically significant decrease compared to the control group ($p < .05$). However, no significant differences were observed among these three groups themselves. At the lower concentrations of 3.2×10^{-6} M and 3.2×10^{-7} M, cell viability was measured as 108.32% and 112.83%, respectively, showing no statistically significant difference compared to the control.

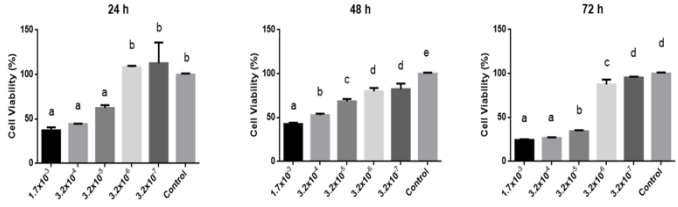

Figure 2.
Cell viability of Thiocolchicoside-treated cells. Columns sharing

the same letters are not significantly different; columns with different letters indicate significant differences between groups according to the Tukey test ($p < .05$).

At 48 hours, the cell viability at 1.7×10^{-3} M concentration was determined as 42.72%, showing a significant decrease compared to the control. For the 3.2×10^{-4} M and 3.2×10^{-5} M concentrations, viability rates were 52.70% and 68.45%, respectively, and both groups showed statistically significant decreases compared to the control and also significant differences between each other. At the lower concentrations of 3.2×10^{-6} M and 3.2×10^{-7} M, viability rates were 79.89% and 82.37%, respectively, showing significant differences compared to the control but no significant difference between each other.

At 72 hours, the cell viability values for 1.7×10^{-3} M and 3.2×10^{-4} M concentrations were 24.42% and 26.73%, respectively, with no significant difference between them but a significant decrease compared to the control. The viability at 3.2×10^{-5} M and 3.2×10^{-6} M concentrations was 34.35% and 87.62%, respectively, with significant differences compared to other groups. The lowest concentration, 3.2×10^{-7} M, showed a viability of 95.45% and did not cause a significant difference compared to the control.

Wound Healing Results

After seeding A549 cells into 48-well microplates, linear scratches were created in each well using a sterile pipette tip 24 hours later. Following this procedure, four different concentrations of Thiocolchicoside—3.2×10⁻4 M, 3.2×10⁻5 M, 3.2×10⁻6 M, and 3.2×10⁻7 M—were applied to the culture media. As shown in Figures 3 and 4:

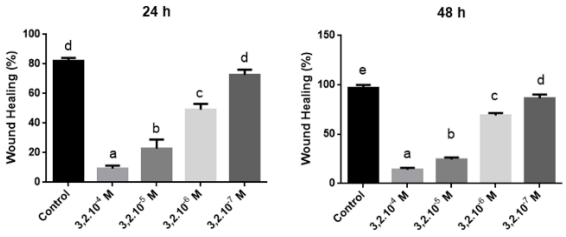

Figure 3.
Wound healing (scratch) analysis.

At the concentration of 3.2×10^{-4} M, wound closure was observed to be 9.25% at 24 hours and 14.07% at 48 hours.

In the 3.2×10^{-5} M group, wound closure was 22.76% at 24 hours and 24.39% at 48 hours. At 3.2×10^{-6} M concentration, closure rates were 49.25% at 24 hours and 69.06% at 48 hours. In the 3.2×10^{-7} M group, wound healing was determined as 72.66% at 24 hours and 86.39% at 48 hours.

Analysis of the 24-hour data revealed no significant difference between the control group and the 3.2×10^{-7} M concentration group, whereas significant differences were observed among the other concentrations and the control group ($p < .05$). According to the 48-hour results, statistically significant differences were found between all Thiocolchicoside concentrations including the control ($p < .05$).

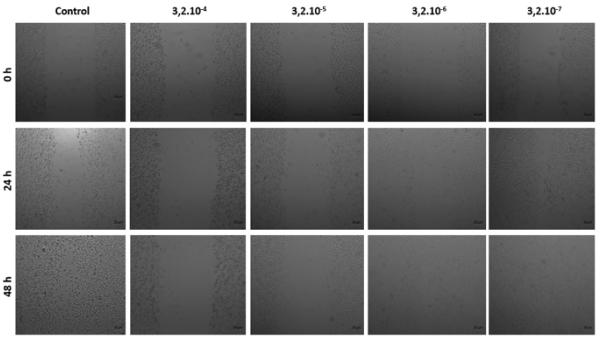

Figure 4.
Percentage of wound healing (scratch). Columns with the same letters are not significantly different; columns with different letters indicate significant differences between groups according to the Tukey test. (p < .05).

Discussion

Cancer is a serious disease that arises from the uncontrolled proliferation of genetically altered cells through evolutionary processes and can spread to surrounding tissues (Brown et al., 2023). Among all diseases, it ranks among those with the highest clinical, social, and economic burdens (Lippi & Mattiuzzi, 2019). Although lung cancer is the second most frequently diagnosed cancer regardless of gender, it remains the leading cause of cancer-related deaths, causing the highest mortality rates among both women and men (Siegel et al., 2023).

In recent years, with the acceleration of technological advancements, interest in researching and developing drugs derived from natural sources has markedly increased. In particular, plant-derived metabolites have been considered to have potential efficacy both for preventive purposes and in combination therapies, gaining increasing importance in this field (Nobili et al., 2009).

Plant-derived compounds have been widely used in traditional medicine for centuries in the treatment of various diseases and have become significant natural products guiding modern pharmaceutical research. Among these compounds, alkaloids are especially noted for their anticancer effects; by disrupting microtubule dynamics, they halt the cell cycle and suppress tumor cell proliferation (Olofinsan et al., 2023). Colchicine is one such plant-derived antimitotic agent, a potent natural alkaloid obtained from plants like Colchicum autumnale or Gloriosa superba. It has been reported that even at nanomolar concentrations, colchicine inhibits the formation of spindle microtubules in the A549 cell line (Bhattacharya et al., 2016).

Thiocolchicoside, a semi-synthetic derivative developed to reduce colchicine's toxicity and enhance its therapeutic potential, is currently used clinically for its anti-inflammatory and muscle-relaxant effects. However, recent studies have shown that

thiocolchicoside may exhibit antiproliferative effects not only on the neuromuscular system but also on cancer cells. Especially due to its inhibitory effects on the NF-κB pathway and activation of tumor suppressor genes such as p53, thiocolchicoside is considered a potential anticancer agent (Simone Reuter et al., 2010). In this context, as a semi-synthetic glycoside derivative of colchicine, thiocolchicoside might exhibit a similar effect profile in A549 cells.

In our study, we evaluated the antiproliferative effects of thiocolchicoside on A549 cells using MTT cytotoxicity assay and wound healing (scratch) analysis. Our results demonstrated that thiocolchicoside exerts cytotoxic effects on A549 cells at high concentrations and inhibits cell migration. MTT analyses revealed that thiocolchicoside decreased cell viability at concentrations of 1.7×10^{-3} M, 3.2×10^{-4} M, and 3.2×10^{-5} M at 24, 48, and 72 hours, indicating a time- and dose-dependent effect of thiocolchicoside. Previous studies on the MCF-7 cell line have shown that thiocolchicoside induces apoptosis by activating the tumor suppressor gene p53 and suppresses the NF-κB pathway (Mahendran et al., 2020). Supporting these findings, numerous studies on other cell lines have demonstrated that thiocolchicoside is a strong inhibitor of NF-κB (Reuter et al., 2010). The suppression of NF-κB increases cancer cell sensitivity by both inhibiting cell proliferation and reducing the expression of anti-apoptotic genes. The findings in these studies support the cytotoxic effect observed in our study and suggest that the possible mechanisms of action may involve these pathways. Future studies can elucidate the molecular mechanisms underlying thiocolchicoside's cytotoxic effect on A549 cells.

In the wound healing (scratch) assay, thiocolchicoside was observed to significantly reduce cell migration, especially at higher concentrations. At concentrations of 3.2×10^{-4} M and 3.2×10^{-5} M, the wound closure rates at 48 hours were only 14.07% and 24.39%, respectively. On the other hand, wound closure at 3.2×10^{-7} M concentration reached 86.39%. These results indicate that the effect of thiocolchicoside on cell migration and invasion processes decreases as concentration increases. It has been reported that colchicine reduces wound healing in A549 cells in parallel with its cytotoxic effects (Balkrishna et al., 2019). Similar effects observed suggest that colchicine is more effective than its semi-synthetic derivative thiocolchicoside at lower doses.

Our study did not evaluate the possible effects of thiocolchicoside on healthy cells besides its selective cytotoxicity on A549 cells. However, literature reports indicate that thiocolchicoside may suppress cancer cell-induced bone loss (Reuter et al., 2012). Therefore, thiocolchicoside could have a significant advantage over other anticancer therapies that cause bone loss. Nonetheless, the lack of expanded biochemical pathway studies, investigations in different cancer cell lines, and in vivo cancer models constitute important barriers for direct clinical translation.

Conclusion

In conclusion, thiocolchicoside showed antiproliferative and wound healing effects on A549 cells that were dependent on time and dose. These findings suggest that thiocolchicoside has anticancer potential and should be supported by further mechanistic studies (e.g., measuring apoptotic protein expression levels, microtubule polymerization analyses, ROS levels). Additionally, investigating the effects of thiocolchicoside on healthy cells both in vitro and in vivo is important to clarify the therapeutic index of this compound.

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