Investigation of the antitumor effects of anti-inflammatory desloratadine and trimebutine on different types of human cancer cells: An in vitro study

Semih Nur Ozkaya\textsuperscript{a}, Tuba Keskin\textsuperscript{a}, Cigdem Tekin\textsuperscript{b}, Suat Tekin\textsuperscript{a,}\textsuperscript{*}, Ali Beytur\textsuperscript{c}

\textsuperscript{a}Inonu University, Faculty of Medicine, Department of Physiology, Malatya, Türkiye
\textsuperscript{b}Inonu University, Vocational School of Health Services, Department of Health Care Services, Malatya, Türkiye
\textsuperscript{c}Inonu University, Faculty of Medicine, Department of Urology, Malatya, Türkiye

Abstract

Aim: Inflammation is a process associated with the development and progression of cancer. Desloratadine (DES) and Trimebutine (TMB) are anti-inflammatory agents used in the treatment of various diseases. This study aimed to investigate the antitumor effects of DES and TMB, which exhibit anti-inflammatory effects, on different human cancer cell lines.

Materials and Methods: In this study, human prostate (LNCaP), ovarian (A2780), breast (MCF-7) and colon cancer (Caco-2) cell lines were treated with DES and TMB at concentrations of 1, 5, 25, 50 and 100 µM. Cells were treated with the compounds for 6, 12, and 24 hours, and the change in cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The inhibitory concentration 50 (LogIC\textsubscript{50}) values of the compounds were calculated using GraphPad Prism 8 software based on cell viability results. The genotoxic effects of the compounds on cells were determined using the comet assay. Group comparisons were performed using the Kruskal-Wallis H test and p<0.05 was considered significant.

Results: Exposure of LNCaP, A2780, MCF-7, and Caco-2 cells to DES and TMB agents for 6, 12 and 24 hours significantly reduced cell viability (p<0.05). According to the comet assay results, DES and TMB caused significant DNA damage in the cell lines (p<0.05).

Conclusion: The study results demonstrate that DES and TMB, which have anti-inflammatory effects, exert cytotoxic effects by inducing DNA damage in cancer cells.

Introduction

Cancer is a disease that is increasingly prevalent and is one of the leading causes of death worldwide [1]. According to research conducted by the International Agency for Research on Cancer, there were 18.1 million new cancer cases and 9.6 million cancer-related deaths globally in 2018 [2]. Studies have reported that cancer occurs as a result of abnormal behaviors of signaling molecules involved in the cellular cycle, such as p53, ERK, and AKT, due to genetic and environmental changes [3, 4]. Novel approaches targeting compounds involved in intracellular signal transmission are important in cancer treatment [5, 6]. It has been observed that anti-inflammatory agents used to reduce inflammation, which is closely related to cancer development [7], induce apoptosis based on the available data [8, 9]. Therefore, the search for effective treatments against cancer has raised the question of whether certain agents with anti-inflammatory roles might have a therapeutic effect. Desloratadine (DES) acts as a histamine H1 receptor antagonist [10, 11] and is commonly used clinically to regulate the inflammatory response in allergic rhinitis [12, 13]. It exhibits a mitigating effect on the inflammatory response by inhibiting the release of histamine [14, 15] and cytokines from mast cells and basophils [16, 17]. A study conducted on human nasal epithelial cells showed that DES could target mediators of the allergic cascade and inhibit the release of chemokines with chemotactic properties, thereby reducing the late-phase response in allergic rhinitis. In the same study, DES was found to exhibit strong anti-allergic and anti-inflammatory properties, which were attributed to the inhibition of ERK1/2 [9]. On the other hand, Trimebutin (TMB) is a pharmacological agent used to accelerate the resumption of intestinal transit and alleviate symptoms associated with irritable bowel syndrome and postoperative paralytic ileus [18].
TMB is also believed to directly affect smooth muscles by blocking voltage-dependent calcium ion (Ca^{2+}) currents and inhibiting the influx and release of Ca^{2+} from intracellular storage compartments [19, 20]. Furthermore, a study examining its anti-inflammatory activity concluded that TMB administered to rats exerted an active effect against inflammation and stress-induced rectal hypersensitivity [21]. Studies in the literature have also shown that TMB can induce apoptosis in a dose-dependent manner in human LOVO colon carcinoma cells and inhibit cell proliferation by inactivating the ERK1/2 signaling pathway [8].

There is limited research in the literature focusing on the effects of DES and TMB on cancer. Considering the influence of the inflammatory response on cancer development and the effectiveness of DES and TMB on ERK, we hypothesized that these two compounds may have anti-proliferative or cytotoxic effects on cancer cells. Therefore, the aim of this study was to determine the cytotoxic and genotoxic effects of DES and TMB on human prostate (LNCaP), ovarian (A2780), breast (MCF-7) and colon cancer (Caco-2) cell lines.

Materials and Methods

Cell culture

The study was conducted at the Department of Physiology, Faculty of Medicine, Inonu University. LNCaP, A2780, MCF-7 and Caco-2 cancer cell lines were used in the study. LNCaP and A2780 cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 5 mL of Minimum Essential Medium (MEM) non-essential amino acids solution. MCF-7 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) High Glucose medium, and Caco-2 cells were cultured in DMEM F-12 medium. Both cell lines were supplemented with 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, 5 mL of MEM non-essential amino acids solution, and 1 mL of insulin. All cells were incubated at 37°C and 5% CO₂ in an Esco CO₂ incubator, with medium changes performed twice a week. Once the cells reached confluence at the bottom of the flasks, they were detached from the flasks using trypsin-ethylenediaminetetraacetic acid (EDTA) solution and stained with 0.4% Trypan Blue. Cell counting was performed under an inverted microscope, and experimental studies were initiated when the cell viability rate reached 90% or above [22, 23]. For cytotoxicity experiments, 96-well plates were used, with 15x10³ cells seeded in each well. After 24 hours of incubation, the cells in the plates were treated with the compounds.

MTT assay method

For the study, stock solutions of DES and TMB were prepared in dimethyl sulfoxide (DMSO) at final concentrations of 1, 5, 25, 50 and 100 µM and added to the wells. After the application of the compounds, the cells were incubated in a CO₂ incubator for 6, 12, and 24 hours. Following the incubation, the effects of DES and TMB on the viability of LNCaP, A2780, MCF-7 and Caco-2 cells were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. This method is based on the ability of the MTT compound to cleave the tetrazolium ring. The compound is absorbed by viable cells and is enzymatically reduced by mitochondrial succinate dehydrogenase, resulting in the formation of a blue-purple, water-insoluble formazan product [24, 25]. This reaction occurs only in cells with active mitochondria, making it a marker of cell viability, and the resulting color is measured spectrophotometrically and correlated with the number of viable cells.

In the MTT analysis, an MTT solution was prepared at a concentration of 0.5 mg/mL in phosphate buffer and filtered through a 0.22 µm filter for sterilization. After treatment with the compounds, the medium in each well of the plates was removed and 50 µL of the prepared MTT solution was added to each well and incubated for 3 hours. After the incubation period, the MTT solution in the wells was removed, and 100 µL of DMSO was added to each well. The optical densities of the cells in the wells were read at a wavelength of 570 nm using an ELISA plate reader (Thermo MultiskanGo, USA) [26]. The absorbance values obtained from the control wells (wells with only medium) were averaged and this value was considered as 100% cell viability. The absorbance values obtained from the wells treated with DES and TMB were normalized to the control absorbance value, and the percentage of cell viability was calculated [23, 27]. The experimental design of the study is shown in Figure 1. These experiments were repeated at least 10 times on different days, independently. After determining the effects of DES and TMB on cell viability through MTT analysis, the inhibitory concentration 50 (IC₅₀) values of these compounds were calculated using GraphPad Prism 8 software and used for comet analysis.

Comet assay method

The comet assay, also known as single-cell gel electrophoresis, is commonly used to assess DNA damage (genotoxicity) [28]. The comet assay technique was performed based...
Table 1. LogIC$_{50}$ (µM) concentrations calculated for LNCaP, A2780, MCF-7, and Caco-2, cells in the Graphpad Prizm 8 program of 6, 12, and 24 h incubation of compounds DES and TMB.

<table>
<thead>
<tr>
<th>LogIC$_{50}$</th>
<th>Compounds</th>
<th>LNCaP</th>
<th>A2780</th>
<th>MCF-7</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES (6 hours)</td>
<td>2.969</td>
<td>2.608</td>
<td>1.944</td>
<td>2.027</td>
<td></td>
</tr>
<tr>
<td>DES (12 hours)</td>
<td>1.677</td>
<td>1.555</td>
<td>1.24</td>
<td>0.6088</td>
<td></td>
</tr>
<tr>
<td>DES (24 hours)</td>
<td>1.211</td>
<td>0.5919</td>
<td>0.6891</td>
<td>0.6308</td>
<td></td>
</tr>
<tr>
<td>TMB (6 hours)</td>
<td>2.291</td>
<td>2.739</td>
<td>1.945</td>
<td>1.639</td>
<td></td>
</tr>
<tr>
<td>TMB (12 hours)</td>
<td>2.391</td>
<td>2.053</td>
<td>1.767</td>
<td>0.8222</td>
<td></td>
</tr>
<tr>
<td>TMB (24 hours)</td>
<td>1.341</td>
<td>1.115</td>
<td>0.4663</td>
<td>0.4865</td>
<td></td>
</tr>
</tbody>
</table>

DES: Desloratadine, TMB: Trimebutine.

The effect of DES and TMB on cell viability

The results of DES and TMB anti-inflammatory agents applied to the LNCaP cell line for 6, 12, and 24 hours are shown in Figure 2. The high dose of DES incubated with LNCaP cells reduced cell viability compared to the control starting from the 6th hour, and this effect became more pronounced over time (p<0.05) (Figure 2.A). Additionally, the applied concentrations of DES were more pronounced at 12 and 24 hours and cell viability started to decrease.

**Figure 2.** Dose-dependent cell viability results of LNCaP cells after 6, 12 and 24 h incubation of DES and TMB anti-inflammatory agents. Each data point is the average of 10 viability measurements. (*) shows p<0.05. (A, DES 6-hour incubation; B, DES 12-hour incubation; C, DES 24-hour incubation; D, TMB 6-hour incubation; E, TMB 12-hour incubation; F, TMB 24-hour incubation; DES: Desloratadine, TMB: Trimebutine).

**Figure 3.** Dose-dependent cell viability results of A2780 cells after 6, 12 and 24 h incubation of DES and TMB anti-inflammatory agents. Each data point is the average of 10 viability measurements. (*) shows p<0.05. (A, DES 6-hour incubation; B, DES 12-hour incubation; C, DES 24-hour incubation; D, TMB 6-hour incubation; E, TMB 12-hour incubation; F, TMB 24-hour incubation; DES: Desloratadine, TMB: Trimebutine).

Statistical analysis

Data were analyzed using the IBM SPSS software program for Windows, version 24.0 (SPSS Inc., Chicago, IL). According to the results of the study, comparisons between groups were made with Kruskal-Wallis H-Test. When statistically significant differences were found between the groups, multiple comparisons were conducted using the Mann Whitney U test with Bonferroni correction. (all values of p<0.05 were considered statistically significant). In addition, according to the MTT assay results, IC$_{50}$ calculations of DES and TMB for all cells were made in the Graphpad Prizm 8 program.
The effects of DES and TMB application on A2780 cells are shown in Figure 3. The high dose of DES applied significantly reduced A2780 cell viability starting from the 6th hour (p<0.05) (Figure 3.A). At 12 hours, the applied concentrations of DES (25, 50, and 100 μM) and at 24 hours, all concentrations of DES caused a significant decrease in cell viability (p<0.05; Figure 3.B and Figure 3.C). After 12 and 24 hours of incubation, the concentrations of TMB at 50 and 100 μM significantly reduced A2780 cell viability compared to the control (p<0.05; Figure 3.E and Figure 3.F). However, after 6 hours of TMB application, the cell viability levels were similar among the groups (Figure 3.D).

The effects of DES and TMB application on MCF-7 cells are shown in Figure 4. The high doses of DES applied from low doses during these time periods. At 12 hours, the cytotoxic effect of DES was significant at doses of 25 μM and above, and at 24 hours, it was significant at doses of 5 μM and above (p<0.05; Figure 2.B and C). The concentrations of TMB applied to LNCaP cells at 50 and 100 μM reduced cell viability compared to the control during 6 and 12 hours of incubation (p<0.05) (Figure 2.D and Figure 2.E). Furthermore, all doses of TMB resulted in significant changes in LNCaP cell viability after 24 hours of treatment (Figure 2.F) (p<0.05).

The effects of DES and TMB application on MCF-7 cells are shown in Figure 4. The high doses of DES applied...
significantly reduced cell viability of MCF-7 from the 6th hour (p<0.05) (Figure 4.A). Additionally at 12 hours, the applied concentrations of DES (25, 50 and 100 µM) and at 24 hours, all concentrations of DES caused a significant decrease in cell viability (p<0.05; Figure 4.B and Figure 4.C). Similarly, we observed the effects of TMB on MCF-7 cell viability starting from the 6th hour. TMB application for 6 and 12 hours at concentrations of 25 µM and above exhibited a significant decrease in cell viability compared to the control (p<0.05; Figure 4.D). After 24 hours of application, all doses of TMB inhibited cell viability compared to the control (p<0.05; Figure 4.F).

The cytotoxic effects determined after applying DES and TMB compounds to the Caco-2 cell line, a colon cancer type, are summarized in Figure 5. The 6-hour incubation of DES with Caco-2 cells reduced cell viability at doses of 50 and 100 µM (p<0.05; Figure 5.A) and we found that the 12 and 24-hour incubations significantly reduced.

The effect of DES and TMB on DNA damage

The levels of DNA damage in cancer cells were evaluated using comet analysis after DES and TMB applications (Figure 6, Table 2). According to the comet results, DES application led to a significant increase in TL, TI and OTM levels in all cell lines except LNCaP cells (p<0.05, Table 2). In LNCaP cells, although an increase in DNA damage was observed after the applications compared to the control, this increase was not statistically significant. After TMB application, the level of DNA damage significantly increased in breast and colon cancer cell lines. However, in prostate and ovarian cancer cell lines, TMB application increased TL, TI and OTM parameters compared to the control, but these changes did not occur at a significant level. Overall, microscopic images indicated that both compounds increased the length of DNA tails in the cell lines (Figure 6). These results demonstrate that DES and TMB can cause DNA breaks in cancer cells, leading to a reduction in cell viability.

Discussion

Cancer is not only a significant public health problem worldwide, but its incidence and prevalence are also increasing. In parallel, research on cancer treatment remains current and relevant [30]. Studies emphasize the importance of new approaches and agents for the treatment of this group of diseases [31, 32]. In our study, we report the potential cytotoxic and genotoxic effects of desloratadine and trimebutine compounds, which exhibit anti-inflammatory effects, on different cancer cell lines. Both compounds demonstrated cytotoxic effects on cancer cell lines depending on time and dosage. We determined that the cytotoxic effect in our study resulted from DNA damage.

In chronically inflamed tissues, disruption of cell death and/or repair programs occurs, leading to uncontrolled replication of cells that have lost normal growth control, including DNA replication. Normal inflammation self-limits because the production of anti-inflammatory cytokines closely follows pro-inflammatory cytokines [33]. However, the inflammatory process seems to positively support cancer development [34]. At this point, anti-inflammatory agents can be included in the treatment against increased inflammation caused by tumors. The strongest evidence for the importance of inflammation during neoplastic progression comes from studies that have identified a reduced risk of cancer in individuals using long-term aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs). These studies report a 40-50% reduction in colon cancer risk and the potential protective effects against lung, esophageal and stomach cancers among individuals using these and similar medications [35].

DES inhibits histamine [14, 15] and cytokines [16, 17] release, thereby reducing the existing inflammatory response. There are studies in the literature indicating the anticancer activity of DES. Fritz et al. reported that DES has the potential to reduce poor prognosis in breast cancer cases and support survival [36]. In their study, Ma et al. investigated the effects of desloratadine on cell growth and invasion in bladder cancer EJ and SW780 cells in vitro. The study results revealed that DES inhibited cell viability in EJ and SW780 cells in a dose- and time-dependent manner. Researchers reported that DES treatment suppressed the colony-forming ability of cancer cells and arrested the cell cycle in the G1 phase in EJ cells. Furthermore, DES treatment promoted cell apoptosis by modulating the expression of Bcl-2, Bax, caspase 3 and caspase 9 in EJ and SW780 cells. These findings indicate a potential anticancer effect of DES on cell growth and bladder cancer invasion [37].

TMB is one of the prokinetic agents primarily used in the treatment of gastrointestinal (GI) disorders such as irritable bowel syndrome. Lacheze et al. reported that trimebutine maleate was effective against local inflammation and stress-induced rectal hyperalgesia in rats [21]. Additionally, there are different studies focusing on the effects of TMB. Jemel-Oualha et al. demonstrated that TMB could induce apoptosis in a dose-dependent manner and reduce cell proliferation by suppressing the ERK1/2 signaling pathway in human LOVO colon carcinoma cells [8]. In another study, the potential anticancer effects of TMB were investigated in SHG44, U251, and U-87 MG human glioma/glioblastoma cells. Fan et al. reported that TMB increased Bax and caspase-3 expression while reducing Bcl-2 expression, thereby promoting apoptosis and significantly inhibiting cell viability [38]. Current studies indicate that DES and TMB activate the mitochondrial apoptotic pathway, leading to cell death and exhibiting anticancer effects in different cancer cells.

In our study, we report the cytotoxic and genotoxic effects of DES and TMB compounds on LNCaP, A2780, MCF-7 and Caco-2 cell lines. DES and TMB applied to cancer cells increased cell death, particularly depending on the treatment duration. The most effective cell death was observed at 24 hours after compound treatment. Additionally, even at low doses, significant levels of cell death were evident during this time period. Our results support the findings in the existing literature that indicate the dose- and time-dependent effects of these compounds. Furthermore, we report in our study that these compounds induce DNA damage in cancer cells. Increased DNA damage results in the activation of molecular pathways leading to
cell death [39]. These findings, in addition to the anti-inflammatory effects of DES and TMB, demonstrate their potential for use in cancer treatment due to their cytotoxic effects on cancer cells.

Acknowledgement
This study was supported by the Scientific Research Projects Unit of İnönü University (BAP; Project No: TSA-2022-2975).

Ethical approval
Ethical approval was not required as it was a cell culture study.

References