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Original Research Article

Evaluation of potential cytotoxic and apoptotic effects of paclitaxel and docetaxel in human Burkitt lymphoma cell lines

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Abstract

Purpose: *To study the cytotoxicity of paclitaxel and docetaxel on cell viability and apoptosis in Burkitt lymphoma cell lines.*
Methods: *The cytotoxic activity of Paclitaxel and Docetaxel against Burkitt lymphoma cells was evaluated using the XTT cell viability test. The cells were exposed to increasing concentrations of paclitaxel and docetaxel ranging from 0.0001 to 10 µM for 48 h. Cell cycle analysis and apoptosis were also assessed using flow cytometry-based experiments.*
Results: *Both paclitaxel and docetaxel exhibited a concentration-dependent cytotoxic effect with IC_{50} of 5.32 and 6.58 µM, respectively. Furthermore, paclitaxel and docetaxel were shown to have a significant apoptotic effect on Burkitt lymphoma cells ($p < 0.01$). Furthermore, paclitaxel and docetaxel arrested Burkitt lymphoma cells at the G2/M phase.*
Conclusion: *Paclitaxel and docetaxel are potential candidates for the treatment of Burkitt lymphoma. Since both drugs are already licensed for many indications, the addition of Burkitt lymphoma to the list of indications of these drugs would be a much easier and faster process. However, there is a need for further in vitro and in vivo research to fully elucidate their mechanisms of action.*

Keywords: *Burkitt lymphoma, Paclitaxel, Docetaxel, Apoptosis, Cytotoxicity*

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INTRODUCTION

Burkitt's lymphoma (BL) is an aggressive type of B-cell non-Hodgkin lymphoma with prominent tumor cell proliferation and is caused by myelocytomatosis (MYC) oncogene translocation. Burkitt's Lymphoma patients who are sufficiently responsive to chemotherapy usually recover by tolerating cures of intensified combination chemotherapy. However, since many treatment approaches have been improved in children and young adults, treatment-related undesirable effects are a significant obstacle, especially in older individuals with comorbidities. To overcome these limiting factors, the search for new agents that can offer alternative options in addition to conventional treatment continues [1].

Paclitaxel, which has a tricyclic diterpenoid

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structure obtained from the bark and needles of *Taxus brevifolia*, is a commonly used anticancer agent with its unique anticancer action [2]. Paclitaxel exerts its cytotoxic effects through microtubule stabilization by causing cellular apoptosis as well as arresting mitosis, thereby inhibiting the growth of cancer cells [3].

With the possible applications of classical drugs in tumor immunotherapy becoming clearer, many studies have shown that this agent directly kills tumor cells and regulates diverse immune cells such as effector T cells, dendritic cells (DCs), natural killer (NK) cells, regulatory T cells (Tregs), and macrophages [4].

Therefore, it is hypothesized that if paclitaxel has a regulatory effect on bone marrow-derived immune cells, including T cells and dendritic cells, it may very well have control over B-cells. So this makes paclitaxel a perfect candidate in the treatment of Burkitt Lymphoma. The current study was aimed to investigate the cytotoxic effects of paclitaxel and docetaxel on cell viability and apoptosis in Burkitt lymphoma cell lines.

METHODS

Chemicals, reagents and cell cultures

Paclitaxel and docetaxel with purity up to 99.5 % were purchased from Sigma (Germany) and a stock concentration (10 µM) in dimethyl sulfoxide (DMSO) was prepared.

Raji cell lines from American Type Cell Cultures were preserved in RPMI-1640 medium (with 10% fetal bovine serum) at 37 °C in a humidified atmosphere containing 5 % CO_2. The cells in the cumulative growth phase were treated with increasing concentrations of Paclitaxel and Docetaxel. The DMSO was used in the control group as a vehicle. The viability of cells at the end of 24 h was determined by the XTT assay with three replicates.

XTT assay

The XTT assay (Biotium, Inc) was used to determine cell proliferation using the manufacturers' guide. To evaluate the cytotoxic effect of paclitaxel and docetaxel on the proliferation of Raji cells, 50 µL of culture containing about 1.5×10^4 cells were seeded to each well of 96-well microplate and incubated with 0.0001, 0.001, 0.01, 0.1, 1 and 10 µM paclitaxel and docetaxel at 37 °C and 5 % CO_2 for 24 h. After incubation, 50 µL XTT labeling solution was added to each well, and then the plates were incubated at 37 °C and 5 % CO_2 for another 4 h. The absorbance of XTT formazan at 450 nm was measured using a spectrophotometer (Thermo, Germany).

Apoptosis assay

The human Burkitt Lymphoma cell line Raji was seeded in a 6-well tissue culture plate (5×10^5 cells/well) and treated with paclitaxel and docetaxel at IC_{50} concentrations (5.32 µM for paclitaxel and 6.58 µM for docetaxel) was incubated for 24 h. After the treatment period, the cells were taken into phosphate-buffered saline (PBS) containing 1 % fetal bovine serum (FBS) and incubated after Annexin V, and Dead cell reagent were added, following the manufacturers' guidelines. Finally, live, dead, early, and late apoptotic cells were detected using the Muse™ Cell Analyzer (Millipore).

Cell cycle analysis

Cell cycle distribution in Raji cells after treatment with 10 µM bioymifi for U266 and 20 µM bioymifi for U266/BR concentrations of 5.32 µM for paclitaxel and 6.58 µM for docetaxel was examined by flow cytometry. After the incubation for 24 h, the cells were harvested, washed with PBS, and fixed in 1 mL cold 70 % (v/v) ethanol. The fixed cells were washed once with PBS and incubated in the darkroom for 30 min with a cell cycle reagent as per the manufacturer's guideline. Finally, different cell cycle stage percentages of the cells were calculated by flow cytometry.

Statistical analysis

GraphPad 7 software was used for statistical analyses of the data and graphical presentations. The data are presented as the mean ± standard deviation (SD). The ANOVA was performed for multiple groups. Statistical significance was assessed at $p \leq 0.01$.

RESULTS

Paclitaxel and docetaxel decreased cell proliferation

As shown in Figure 1, there was a significant and concentration-dependent decrease in the proliferation of Raji cells treated with paclitaxel and docetaxel for 48 h compared to that of the untreated cells. The IC_{50} values of paclitaxel and docetaxel in Raji cells were recorded as 5.32 and 6.58 µM, respectively.

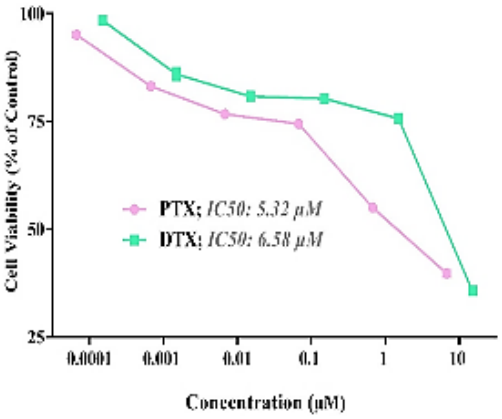

Figure 1: Paclitaxel and docetaxel treated Raji cells. (n = 3, $p \leq 0.01$)

Effect of paclitaxel and docetaxel on apoptosis

Apoptosis in Raji cells was assessed following paclitaxel and docetaxel treatment. As seen in Figure 2, 5 µM of paclitaxel and docetaxel for Raji cells activated a noticeable apoptotic effect compared to the untreated cells ($p < 0.05$). The total apoptotic cell population was defined to be 22.7 % in the paclitaxel group and 15.8 % in the docetaxel group.

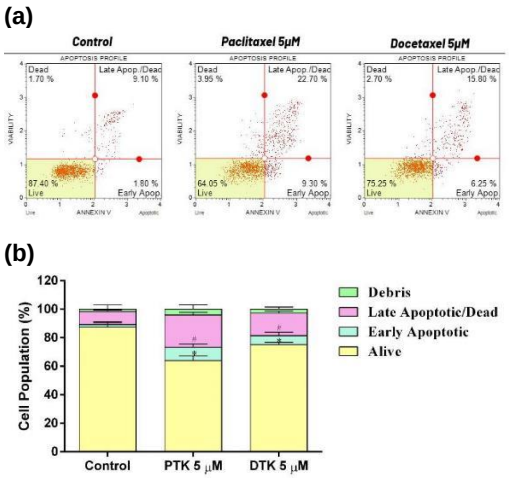

Figure 2: Apoptotic effects of paclitaxel and docetaxel on Raji cells treated with IC_{50} (5 µM of paclitaxel and docetaxel) values for 48 h. (a) Results of flow cytometric analysis of Raji cells; and (b) Percent apoptotic/non-apoptotic cells in the bar graphs. $P \leq 0.01$ when compared to the control cells

Cell cycle arrest in G2/M mitotic phase

To determine whether the anti-proliferative role of paclitaxel and docetaxel against Raji cells was due to the induction of cell cycle arrest, Raji cells were incubated with 5 µM concentrations of paclitaxel and docetaxel, and cultured for 48 h at 37 °C. Next, the effect of paclitaxel and docetaxel on cell cycle progression was detected using the cell cycle kit. Flow cytometry results indicated that treatment with paclitaxel and docetaxel caused the Raji cell population to increase in the G2/M phase (Figure 3).

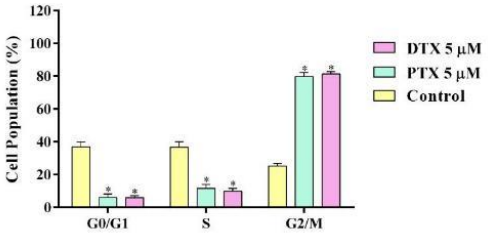

Figure 3: Effects of Paclitaxel and Docetaxel on cell cycle distribution. The histogram displays the percentage of cell cycle phases of Raji cells

DISCUSSION

Paclitaxel, obtained from the bark of the yew tree *Taxus brevifolia* Nutt, is the most widely used anticancer agent against many types of cancers in recent times [5]. It is a compound belonging to the diterpene taxane class, which reveals its anticancer effect with its microtubule stabilization mechanism [6,7]. It is known that the compound obtained by the extraction method has strong anticancer activity against many malignancies as well as breast cancer. In addition, increasing evidence for cancer research indicates the use of a semi-synthetic taxane analog synthesized from the European yew tree *Taxus baccata*. Although the structure of the compound changed with a very small difference, the remarkable changes observed in favor of physicochemical and biological properties increased the clinical applicability of the compound [8].

The anticancer effects of both paclitaxel and docetaxel have been shown in the literature. Taxanes used in the present study inhibited the cell proliferation of Raji cells. The apoptotic results of paclitaxel and docetaxel have been shown in this study. Annexin V staining after taxane administration was significantly higher when compared to the control group. This finding is also in line with the literature [9]. It has been shown that paclitaxel can stimulate apoptotic modulator genes, which reveals that paclitaxel is independent of microtubule maintenance. It has been speculated that this apoptotic effect might be related to regulating the transcription of genes related to inflammation and DNA damage response proteins, proteins involved in the regulation of apoptosis and cellular proliferation, and cytokines [9].

Furthermore, one of the main features of paclitaxel and docetaxel is to induce cell arrest in

the G2 phase. It was shown by Guo *et al* [10] that paclitaxel-induced G2/M phase arrest in hypoxia-resistant Non-small-cell-Lung Cancer cells was hampered, but FV-429 improved the sensitivity of these cancer cells to paclitaxel. Similarly, Li *et al* [11] showed that Tn-Lipo-PTX could efficiently increase the percent of HepG2 liver cancer cell arrest in the G2/M phase. In the present study, it was demonstrated that both paclitaxel and docetaxel arrested the cell cycle of Raji cells in the G2/M phase.

CONCLUSION

The results indicate that both paclitaxel and docetaxel have anticancer effects on Raji Burkitt Lymphoma cells. This antiproliferative effect is based on microtubule inhibition along with apoptosis and cell cycle arrest. When the immunomodulatory effects of these drugs are considered, they are potential candidates for the treatment of Burkitt lymphoma.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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