

The evaluation of Paclitaxel effects on the Caco-2 cell line of colon cancer patients

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Abstract

Background: Colon cancer is considered a malignant neoplasm. Paclitaxel is anti-carcinogenic chemotherapy that stimulates the apoptosis of cancer cells.

Objective: This study aims to know the effect of paclitaxel on the level of Reactive oxygen species (ROS) on Caco-2 of colon cancer cells by the High-content screening technique.

Methods: Normal and cancerous human colon cells (Caco-2 cell lines) were cultured, then treated with paclitaxel at two different quantitative doses (100 µg/ml) and (200 µg/ml) for 1-7 days. The examination was carried out at the University of Malaya Kuala Lumpur, College of Medicine, specifically at the Natural Products Research Center and the Department of Drug Discovery in Pharmacology in Malaysia.

Results: The high-concentration paclitaxel doses ($P \Rightarrow 0.0001$) were more effective than the low-concentration paclitaxel doses ($P = 0.0043$) when compared with the healthy person as the high dose had a significant effect in inhibiting the growth of cancer cells on days 7 by stimulating ROS secretion in the cells. The epithelium of the colon (CaCO-2). High-content screening used to detect oxidative stress.

Conclusion: Higher doses of paclitaxel are more effective over time to inhibit the growth and spread of colon cancerous cells and reduce the effects on normal colonic epithelial cell growth. The increased level of ROS in the Caco-2 cell line appears to be associated with the increased dose concentration of paclitaxel.

Keywords: Colon cancer, Reactive oxygen species, Paclitaxel, oxidative stress.

INTRODUCTION

Colon cancer is one of the malignant diseases prevalent among women and men, ranking fourth in the world as a cause of death and in men, it ranks third after lung and prostate cancer, and in women, it ranks second after breast cancer [1]. There is an expectation of an increase in the incidence of colon cancer by the year 2030, with about 2.2 million patients and 1.1 million deaths [2]. Colon cancer has three types: the first type is sporadic, as it is diagnosed in about 70% of colon cancer patients [3]. The second type is hereditary and diagnoses about 5% and the third type is familial and constitutes about 25% of colon cancer patients [4]. The colon cancer carcinogenic process has three pathways, including chromosomal instability (CIN) that occurs as a result of a defect in the separation of DNA chromosomes upon reproduction and the island methylation phenotype (CPG) that occurs due to epigenetic instability (genes lose expression of proteins) and microsatellite instability (MSI) that occurs due to a defect in the mechanism of DNA repair [2]. Treatment strategies for colon cancer patients depend on the size, location, and extent of the tumor, and the treatment is either surgical (local or partial removal) or chemotherapy or radiotherapy or all of them [5]. Chemotherapy is an anticancer drug used before or after surgery [6]. 5-Fluorouracil is the mainstay of chemotherapy in colon cancer patients. It is often used in combination with Taxol to enhance the sensitivity of cancer cells to the drug [7]. Increased production of reactive oxygen species (ROS) as a result of a defect in the reducing factor or due to genetic mutations or oxidative stress, which are free radicals that contribute to the carcinogenesis and spread of colon cancer [8]. Therefore, the treatment of colon cancer is often drugs that control the level of ROS in cancer cells [9]. Paclitaxel (PTX), commercially known as Taxol, is an anticancer drug that promotes apoptosis of cancer cells and is used to treat breast, colon, lung, and other cancers [10]. Paclitaxel is one of the most successful natural medicines, which is produced from the bark and needles of Taxol, its molecular formula is C₄₇H₅₁NO₁₄, which consists of three diterpenoid rings, which has an important role in the immunity of

cancer cells, so it is one of the most widely used and successful medicines [11]. Caco-2 cell line is a human epithelial cell line used as a model for the intestinal epithelial barrier where it is derived from colon cancer and it is also the main cell line for discovering how drugs work due to its ability to differentiate into a monolayer composed of many cells in addition to expressing the characteristics of intestinal cells [12]. Use High-content screening or cytology is a powerful technique used in drug discovery processes combining the quantitative analysis of the images obtained from the automatic microscopy analysis [13]. This technique can also be used to detect oxidative stress through the use of fluorescent probes [14]. The main objective of this study is to elucidate the ability of paclitaxel to generate ROS in the Caco-2 cell line and its relationship to colon cancer development by way of the exit of an electron from the mitochondria leads to the formation of free radicals.

Materials and method

Materials

MnSOD and induction assay phospho-H2AX originating from Thermo Scientific, USA.

When using the HCS assay to determine the amount of ROS produced by living cells, use Hoechst's fluorescent dye.

Method

This case control study includes two samples (1 patient and 1 control) were collected from Baghdad Teaching Hospital, Baghdad, Iraq. A Caco-2 cell line was taken from patient with colon cancer and a healthy. Iron citrate was diluted to (400 µg/ml) and paraquat was diluted to (1 mM) using a culture medium and then 100 µl of the solution was added to all wells of the plate and then incubated in the incubator at 37°C for 20 hours. Paclitaxel at different concentrations (100 µg/ml, 200 µg/ml), (100 µl/well) was added to the wells followed by further treatment and serial dilution and then incubated at 37° for complete. per day 5% of carbon dioxide.

The examination of the reactive oxygen level after using the drug Paclitaxel was done at the University of Malaya Kuala Lumpur, College of Medicine, specifically in the Natural Products Research Center and drug discovery Department of Pharmacology.

The Caco-2 cell line was cultured in a culture medium containing 10% heat-inactivated FBS, 1% glutamine, non-essential amino acids, and 0.5% penicillin/streptomycin to complete the process of cell proliferation and distribution in plate wells [15].

Statistical analysis

Statistical analysis was performed using Graph Pad Prism Edition 6 (Graph Pad Software Inc., La Jolla, CA). The results were expressed as mean, standard deviation (mean ± SD). P values of 0.05 were considered statistically significant.

Result

It was found when using paclitaxel drug to treat Caco-2 cell line for the colon cancer-patients to assess reactive oxygen species after 24-hour exposure to paclitaxel using the HCS and use the untreated cell line as a negative control. When using (100 µg / mL) of the drug, the mean ± standard deviation was equal to (721.3 ± 36.47) and the significant value compared to the control was (P-value = 0.0043), however when using (200 µg/ mL) From paclitaxel drug, the mean ± standard deviation was (1222 ± 133.2) and the significant value compared to the control was (P-value = > 0.0001) as shown in the figure 1.

In Figure 2, we notice that the HDE stain detects oxidative stress, the green color for the full detection of the ROS detector and the superoxide detection detector is the blue color. By combining the two fluorine probes, they provide a simple and specific method. This assay is designed to directly monitor ROS production in live cells.

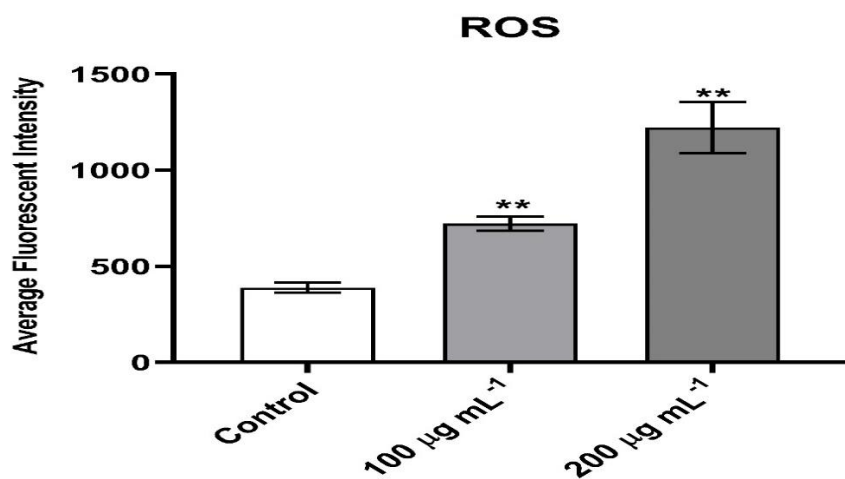


Figure 1: Paclitaxel effect on the induction of ROS in a Caco-2 cell line (after 24 hours of incubation at 37°C). The test has been improved by using the reader to examine the thermal HCS scientific matrix.

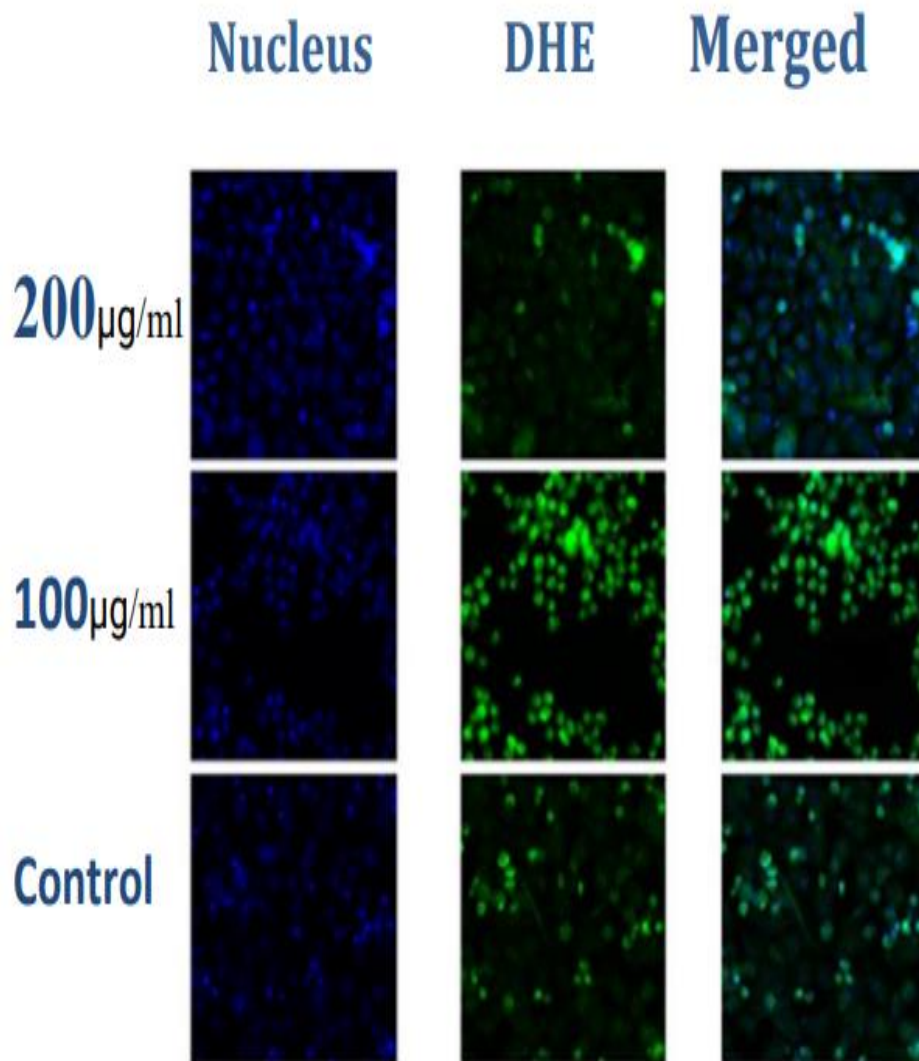


Figure 2: Dealt with the intensity of the fluorescent dye DHE different concentrations of paclitaxel.

Discussion

It has been noticed through the results that were obtained that the production of ROS in cancer cells increases in the level of ROS by approximately 85.5%, when using (100 µg / ml) of the drug concentration, we also noticed an increase in reactive oxygen production of about 214% when using (200 µg/ml) of the drug and these results indicated that paclitaxel increases ROS production in the Caco-2 cell line. The limitations of using Caco-2 cell line model is that; when compared with the normal intestinal epithelium containing different types of cells that the Caco-2 cell line does not contain mucus and an un-inverted aqueous layer. Thus, this affects the uptake of some compounds by cells. Given the cost of an HCS examination, a few samples were determined and the relationship of PTX to ROS level was confirmed. The level of ROS could be related to the body mass index and age of the patients. In conclusion, the use of paclitaxel in the treatment plan for colon cancer patients helps to reduce the spread of cancerous tumors by increasing the generation of free radicals. In this study the increase in the level of ROS may be due to stimulation by paclitaxel to increased Rac1 translocation leading to activation of membrane-bound activation of nicotinamide adenine dinucleotide oxidase (NOX) which is consider the main source of superoxide (O₂) which turns into hydrogen peroxide (H₂O₂) by superoxide dismutase (SOD) and leads to chromosomal instability, and all these lead to apoptosis and making microtubule dissociation difficult [16, 17]. therefore, it was stopping the cell cycle by preventing cell division in the G₂/M phase and prevents cancer cells from multiplying and spreading [18, 19]. Paclitaxel is used to control the level of reactive oxygen species in cancer cells at the stage of chemotherapy, in early cases after surgical removal, and in advanced stages of colon cancer [20]. Paclitaxel is given to patients with colon cancer at different doses according to the therapeutic approach to chemotherapy [21]. Our results are in agreement with the results of the research presented by Yun-Chi Tang (2017), which focused on the effect of Taxol on the occurrence of nuclear changes in cancer cells, genomic instability, protein, and energy toxic stress that induces the death of cancer cells [22]. It also agreed with the results of the research presented by Duy Toan Pham (2019), where it was used with Taxol silk fibroin to facilitate drug delivery to Caco-2 cells while maintaining the drug's efficacy in stimulating apoptosis [23]. In conclusion the Paclitaxel drug increase the reactive oxygen species induction of Caco-2 cell for colon cancer patients.

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Conflict of interest

The authors state that there are no conflicts of interest to disclose.

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Author contributions

The current study was drafted by Prof. Dr Firas A. Hassan is responsible for analyzing the data. Sana was responsible for collecting samples and writing the report and the tissues were cultured in Malaysia

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