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High Concentration of Paclitaxel Induces Partial Inhibition and Cell Death in the Human Breast Cancer Cell Line MCF-7 In-Vitro

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ABSTRACT

Purpose: Cancer remains one of the most common causes of death and thus one of the goals of cancer research has been and continues to be, the discovery of natural and synthetic products for cancer prevention and for treatment. For time immemorial, plants played a dominant role in the treatment of human ailment. Therefore, in the present study we examined the in vitro effect of paclitaxel on the growth rate and morphology of the human mammary tumor cell line (MCF-7).

Material and Methods: MCF-7 cells were counted and cultured under aseptic conditions. Paclitaxel was added to cultured cells at different concentrations, 0.5, 2, 5, 7 and 10 µg/ml. Both treated and untreated cells were harvested and tested for viability.Apoptosis was detected by using DAPI fluorescent dye and DNA fragmentation assay.

Results: Our results showed that paclitaxel at a concentration of 5µg/ml inhibited in vitro tumor growth significantly within a period of 48 hours Microscopic examination of the cell morphology as well as nuclear staining revealed that the paclitaxel treated-cells suffered dramatically from membrane swelling, disruption and nuclear fragmentation.

Conclusion: Necrosis and apoptosis seem to be the underlying mechanisms by which paclitaxel affect human mammary tumor cells in vitro.

Key Words: *Breast cancer - MCF-7 - Apoptosis - Necrosis - Paclitaxel.*

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INTRODUCTION

Cancer is considered one of the most common causes of morbidity and mortality all over the world. Breast cancer is the second cause of cancer deaths among women. The report published by the American Cancer Society estimates that about 43,700 women and men will die because of breast cancer in the United States [8].

Thus, the target of most researches has focused on discovery of natural and synthetic compounds that can be used in the prevention and/or treatment of cancer. Many of the natural products that exhibit anti-tumor activity have been discovered [20]. Gossypol, a spermatoxin derived from crude cotton seed oil, seems to be inhibitory to the growth of adrenocortical tumor cells in nude mice as well as to adrenal cancer in human [11]. The plant toxin Saporin, a ribosome inactivating protein, has been also shown to inhibit the growth of both normal and tumor cells in vitro [3].

Recent studies have indicated that paclitaxel (Taxol), a naturally occurring antimitotic agent, has a significant cell-killing activity in a variety of tumor cells in combination with other agents [1,12]. The initial clinical trials with paclitaxel/ cisplatin demonstrated a marked anti-tumor efficacy of this combination in ovarian and breast cancer [9,14]. Subsequent clinical trials expanded the experience with this agent showing that paclitaxel retained substantial antitumor effica-

cy in previously treated patients and that paclitaxel was active against metastatic breast cancer at a variety of doses and schedules of administration [22].

Comparative clinical trials to determine the optimal dose and schedule of administration of this agent are completing accrual. Paclitaxel has been extensively evaluated in combination with other cytotoxic agents which demonstrated activity against metastatic breast cancer. The mechanism by which paclitaxel induces cell death is not entirely clear [15].

Several reports suggest that the paclitaxel enhances the apoptotic pathway which leads to the death of cells. The morphologic changes associated with apoptosis include chromatin condensation and nuclear pyknosis; fragmentation of the nucleus and formation of apoptotic bodies consisting of membrane-enclosed pieces of condensed chromatin and well-preserved organelles [2,24]. Another characteristic change occurring in apoptosis results from endonuclease activity that cleaves transcriptionally active nuclear DNA [18], which can be detected by changes in cell morphology [19].

So, this study was directed to examine the in-vitro effect of paclitaxel as a single agent on human breast cancer cell line (MCF-7) in order to figure out its action.

MATERIAL AND METHODS

Cell line:

Human breast cancer cell line MCF-7 (kindly provided by Dr. Nural H. Sarkar, Medical College of Georgia, USA) was maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2mM L-glutamine hydrocortisone, 1% streptomycin/penicillin and 10% fetal calf serum at 37ºC in a 5% CO_2 incubator. One week before treatment with paclitaxel, 5% charcoal-dextran treated calf serum was added to the medium.

Treatment with paclitaxel

Cells were cultured in 6 flasks. The first five flasks were treated with paclitaxel at concentrations of 0.5, 2, 5, 7, and 10 µg/ml, respectively. RPMI containing cremophore was added to the sixth flask instead of paclitaxel and considered as a control. The viability and the morphology of the cells were checked after 12, 24, 48 and 72 hours from the treatment by using trypan blue [5].

Morphological evaluation of apoptosis:

Morphological changes in the nuclear chromatin of cells were detected by staining with 4,6-diamidino 2-phenylindole (DAPI) for 30 minutes. After fixation with freshly prepared Carnoy's solution, the slides were washed twice with running water, air-dried and examined under a fluorescent microscope. Cells were counted and scored for the incidence of apoptotic chromatin changes [13].

Fragmentation assay:

DNA was extracted from MCF-7 cells by suspending in 100 µl of 10 mM tris-HCl, 10% SDS and 10 mM EDTA (pH 8). Then, 200 µg/ml proteinase K were added and incubated at 37ºC for 3 hours, followed by extraction with phenol/chloroform. DNA was precipitated with 100ml of 7.5 M ammonium acetate and 3 volumes of ethanol. DNA prepared from control cells and from paclitaxel-treated cells at different times were analyzed by electrophoresis for 14 hours at 20V on 1.0% agarose gel [10].

RESULTS

Paclitaxel-induced death of MCF-7 cells in vitro:

Paclitaxel showed a significant inhibitory effect on the growth and viability of MCF-7 cells in vitro. This inhibitory action was noticed to be dose and time dependent. The maximum inhibitory effect of paclitaxel was obtained at a concentration of 5 µg/ml of culture media. On the other hand, there was no statistically significant change in the viability of MCF-7 cells after 12 and 24 hours of treatment with 5 µg/ml of paclitaxel, where the percentages of dead cells were 9.1% and 18.4%, respectively. On the other hand, after 48 hours, the percent of cell death was significantly increased to 42.2%. At the same time, no more changes were observed after 72 hours of treatment with paclitaxel (Fig. 1-A, B).

Cell morphology analysis:

Microscopic examination showed no differences in the morphology and pattern between the control cells and cells after 12 or 24 hours of paclitaxel treatment. The cells were colonized at the beginning of culture and then

formed a monolayer attached to the bottom of the flask. After 48 hours of paclitaxel treatment, the cells were rounded up and the nucleus became phase-dense and most of cells were detached. At the same time, the cells did not exhibit condensation of apoptotic bodies (Fig. 2).

Histochemical staining of apoptotic cells:

After 48 hour-treatment of MCF-7 cells with 5µg/ml of paclitaxel, nuclear structure using DAPI staining exhibited condensation and fragmentation of some nuclei, as shown in fig. (3).

DNA laddering:

Fig. (4) shows the pattern of DNA isolated from MCF-7 cells after treatment with 5 µg/ml of paclitaxel. Electrophoresis of DNA obtained from control cells as well as from cells treated with paclitaxel for 12, 24, 48 and 72 hours were found to be intact.

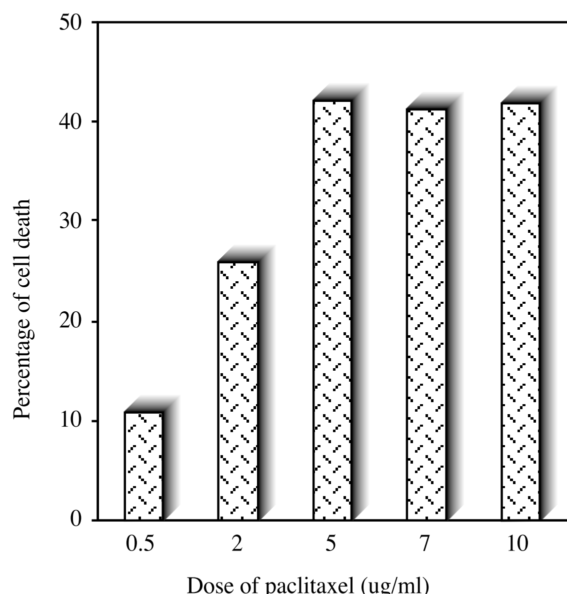


Fig. (1-A): Dose effect of Paclitaxel on the viability of MCF-7 cells.

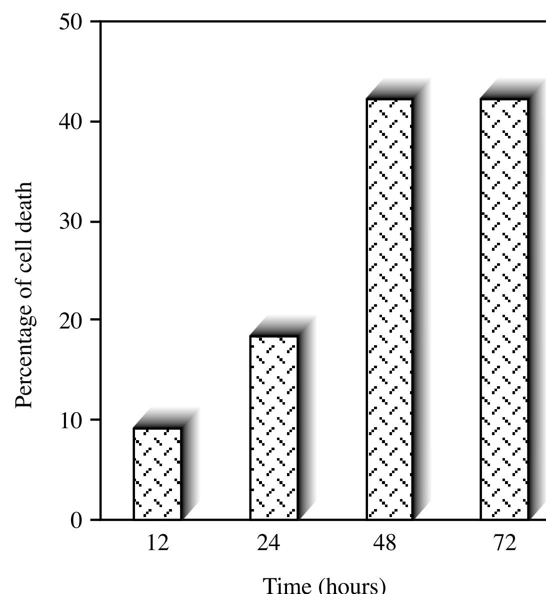
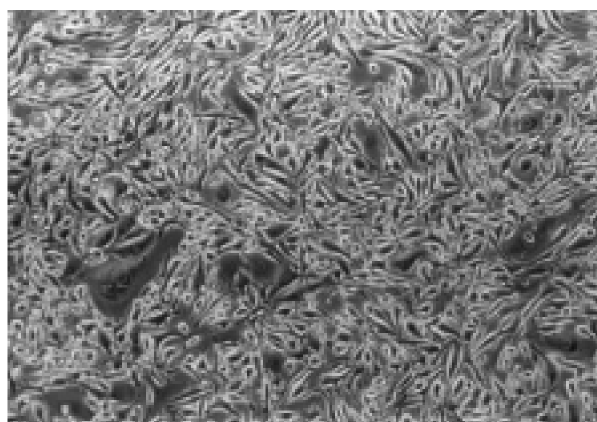
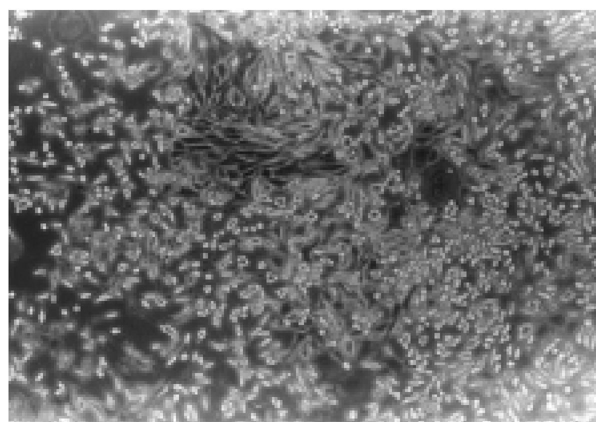


Fig. (1-B): Timing effect of 0.5 mg/ml paclitaxel on the viability of MCF-7 cells.

Fig. (2): Phase contrast light micrograph of MCF-7 cells.



(A): untreated cells.



(B): MCF-7, 48 hr after treatment with 5mg/ml of paclitaxel.

Fig. (3): Apoptosis in humans MCF-7 cell line.

(A): untreated cells.

(B): MCF-7, 48 hr after treatment with 5mg/ml of paclitaxel.

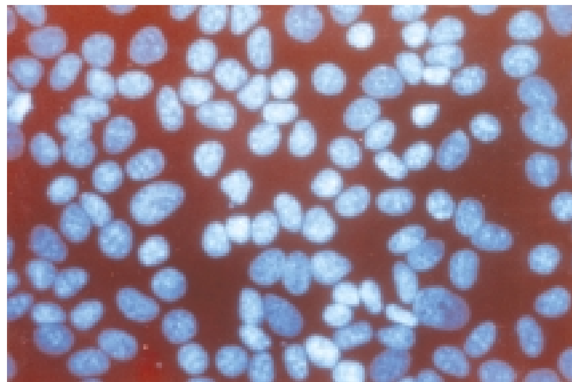
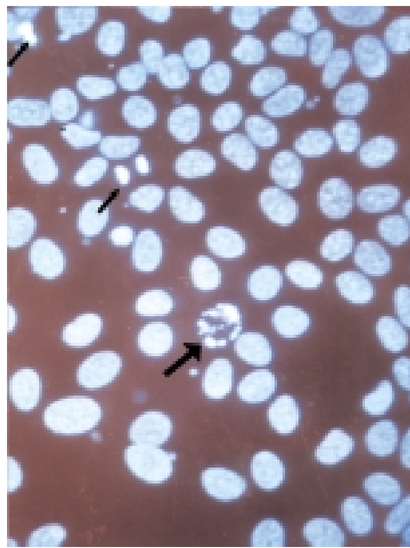


Fig. (3-A)



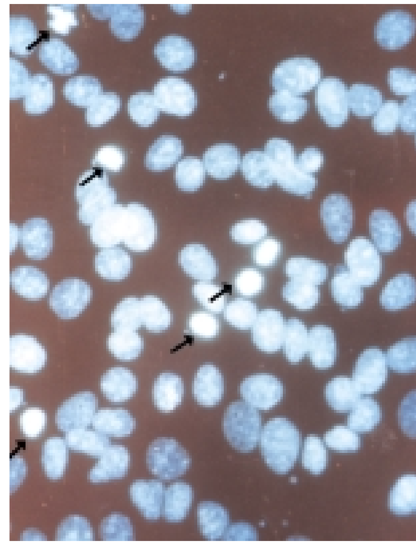


Fig. (3-B)

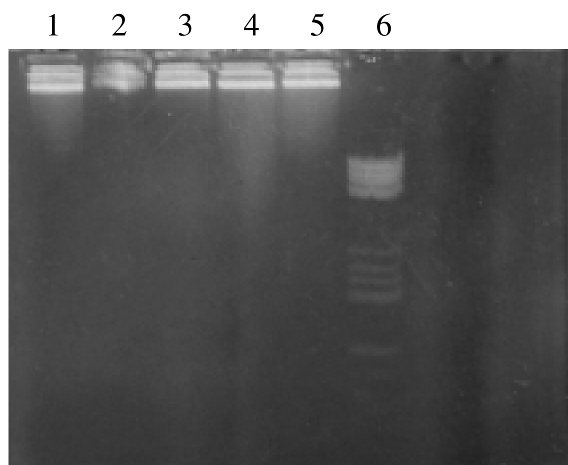


Fig. (4): Electrophoretic analysis of DNA isolated from MCF-7tumor cells.

- Lane 1: DNA of untreated cells.
- Lane 2-5: DNA of MCF-7 after 12, 24, 48 and 72 hr of treatment with 5mg/ml.
- Lane 6: 100-base pair DNA ladder as a molecular weight marker.

DISCUSSION

Although paclitaxel has emerged in the last 10 years as a successful drug in cancer therapy, the overall response rate to this drug in patients with advanced metastatic disease remains low. Therefore, an understanding of the dose, duration and whether paclitaxel should be used alone or in conjunction with other compounds is critical for improving the therapeutic efficiency of this drug.

The current findings demonstrated that paclitaxel (0 to 5 µg/ml) caused concentration-dependent increase in morphologically identifiable apoptotic cells (up to 42.2% of cell population). At the same time, maximal apoptotic-appearing MCF-7 cells occurred after 48 hours of exposure to paclitaxel. Comparable findings were reported in the study of Saunders et al. **[21]**, where they found that 0 - 20 ng/ml of pac-

litaxel caused concentration-dependent increase in apoptotic cells (up to 43% of cell population). Maximal apoptotic-appearing MCF-7cells occurred after 24 hr of exposure to paclitaxel.

On the other hand, histochemical analysis of the MCF-7 tumor cells treated with 5mg/ml of paclitaxel for 48 hours revealed the presence of only a small population of tumor cells (up to 10%) with fragmented nuclei and apoptotic bodies. Furthermore, the genomic DNA of the paclitaxel-treatedcells appeared to be intact and did not exhibit any fragmentation. Taken together, these findings suggest that paclitaxel exerts its cytotoxic effect mainly through cell necrotic mechanism and sometimes apoptosis. One of the most reliable criteria for distinguishing between apoptosis and necrosis is cell morphology. In this study, the death of target cells could be assigned morphologically to necrosis by phase contrast microscopy (Fig. 2).

Despite numerous studies that have examined paclitaxel-cell death, it remains uncertain whether cells die through necrosis, apoptosis or both **[7,17,23]** and what the underlying mechanisms are. However, it should be noted that conditions that induce apoptosis are cell type-dependent **[4]**. It has been reported recently that the widespread use of MCF-7 human breast cancer has led to variation in these cells between different laboratories; differences in sensitivity to apoptosis have just begun to be described **[6]**.

One of the purposes of tumor therapy is retarding growth and preventing proliferation, which has been achieved in this work. It is clear that paclitaxel affects tumor cells when used as a single agent without combination with other chemotherapeutic agents to avoid markedly different toxicities.

For the time being, necrosis is mainly the underlying mechanism by which paclitaxel affects tumor cells, in the presence of apoptosis. Presently, we do not know how paclitaxel induces necrosis. Further studies should be done to drive more information about the inhibitory effect of paclitaxel on tumors in vivo and to optimize its action and concentration in biological systems in the coming years in order to extend the knowledge for using different anticancer drugs and/or compounds from a natural source.

Conclusion:

From the present study, we can conclude that paclitaxel partially inhibits human breast cancer MCF-7 cells without combination with other chemotherapeutic agents. At the same time, the study has clearly demonstrated that paclitaxel affects tumour cells mainly via necrosis and to a lesser extent, apoptosis. Further studies should be performed on experimental animals to spotlight the mechanism of action of paclitaxel at the molecular level of the tumor cells.

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