Journal of US-China Medical Science17 (2020) 75-79
doi:10.17265/1548-6648/2020.02.004

Investigation of the Effect of Paclitaxel on MCF-7 on Breast Cancer Cell Line

Mustafa Nisari

Department of Nutrition and Dietetics, Faculty of Health Sciences, University of Nuh Naci Yazgan, Kayseri38090, Turkey

Abstract: The aim of this study is to investigate the effects of different doses of paclitaxel against *in vitro* cell proliferation of the human breast cancer cell line MCF-7.MCF-7 cell line was used. It was divided into 4 groups, one control and three experimental. 10 µg, 100 µg and 1000 µg paclitaxel were applied to the culture medium belonging to each group, respectively. Later, viability and/or proliferation of the cells were determined by MTS test. An increase was observed in the experimental group cells depending on the dose. The average number of cells obtained from the control and experimental groups was 48.4×103 (±1.6), 21.6×103 (±1.08), 17.4×103 (±1.12) and 11.4×103 (±0.79), respectively. The decrease in the experimental groups compared to the control group was statistically significant (p<0.05). When the experimental groups were compared, the number of cells at high dose was low and was statistically significant (p<0.05). In our study, the apoptotic effect of paclitaxel was demonstrated in the MCF-7 cell line. We believe that paclitaxel used in the treatment of cancer diseases will contribute to the reliability.

Key words: MCF-7, angiogenesis, paclitaxel.

1. Introductıon

Angiogenesis is the formation of new capillaries from pre-existing blood vessels and is a basic process involved in various physiological and pathological processes [1]. The growth and metastases of solid tumors depend on the induction of adequate blood flow [2]. Angiogenesis has become a very promising target for experimental treatments in cancer and a wide variety of treatments are being developed to interfere with this process [3]. Cancer chemopreventive agents inhibit cell growth, proliferation, and induce apoptosis in various cancer cell lines, and blocking angiogenesis provides a new therapeutic target against tumor spread. Some chemopreventive agents, such as genistein [4] and curcumin [5], inhibit angiogenesis in a variety of *in vitro* and *in vivo* models. Thus, it may be of great clinical importance to identify chemopreventive agents with multiple biological activities that are nontoxic and act at various stages of angiogenesis [6].

Various chemotherapeutic drugs have been investigated for anticancer activity. Paclitaxel, also known as taxol, is a chemotherapeutic drug with a high medicinal property and is one of the most frequently used chemotherapeutics in the clinic. It is a broad spectrum anticancer drug that is effective in various solid tumors such as ovarian and breast cancer, lung cancer, melanoma, head and neck cancer, and bladder cancer. Paclitaxel has a strong anti-proliferative effect against tumor cells and by stabilizing microtubules, blocking mitosis and inducing apoptosis. Angiogenesis involves a complex sequence of events. With angiogenic stimulation, vascular endothelial cells stimulate cell proliferation to break up the extracellular and tissue matrix, increase endothelial cell motility and provide the required number of cells for growing vessels [7]. The aim of this study is to investigate the effects of different doses of paclitaxel against *in vitro* cell proliferation of the human breast cancer cell line MCF-7.

Corresponding Author: Mustafa Nisari, Dr., Asst. Prof.,research fields:biochemistry, cancer.

2. Materıal and Method

2.1 Cell Culture

In the study, MCF-7 cells (Cell Line humanfrom human breast (adenocarcinoma), Merck; 86012803) planted from a commercially produced cell line were used. MCF-7 in 5% CO_2 humid incubator at 37 °C in medium containing streptomycin/penicillin (100 U/mL; Sigma, St. Louis), 10% fetal bovine serum (Sigma, St.Louis) and RPMI 1640 was cultured. Healthy MCF-7 cells were then grouped for paclitaxel administration.

2.2 Experiment Groups

After the cells were counted in Thoma slide, they were divided into 4 groups as 1 control and 3 experimental. In each well were cultivated 5×104 cells forming the groups. After the cells planted in culture wells of 96 wells were kept in an incubator overnight, the wells were washed twice with PBS and the medium was replaced. After incubating for 48 hours, only the fattening was given to the control group, while 30 wells containing 10 µg, 100 µg and 1,000 µg paclitaxel were added to the wells forming the experimental groups. Then, to count the endothelial MCF-7 cells, the cells in the culture dish were washed three times with PBS, and 20 µL of trypsin/EDTA was added and kept at 37°C for 1-2 minutes. Then 40 µL medium was added and centrifuged (this process was done twice) for 10 minutes at 1,000 rpm. Twenty (20) µL of medium was added to the pellet remaining at the bottom of the eppendorf and the cells were suspended with a pipettor. Ten (10) µL of the suspended mixture was taken and placed in another eppendorf tube. Pipetting was done by adding 10 µLtrypan blue. Took 10 µL of the suspension we prepared, transferred to Thoma slide and counting area was found by adjusting the microscope lens to ×40 and cells were counted.

2.3 MTS Analysis

The viability and/or proliferation of cells were detected by MTS assay (Promega, Madison, WI, USA), after cells treatment, to measure cell growth. Cells were counted using a hemocytometer and viable cells were identified by trypan blue exclusion. Viable cells were seeded in 96-well plates (5×104 cells/well), and transfected with indicated 10 µg, 100 µg and 1,000 µg paclitaxel. After 72 h of treatment, a solution containing MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) and PMS (phenazine methosulfate) (20:1 *v*/*v*) was added to the cells. After 2-3 h of incubation at 37°C, the viable growing cells were estimated by monitoring the absorption of the product at 490 nm, based on the generation of formazan via live cells. All experiments were performed in triplicate and the results were reported as mean of absorption±standard deviation.

2.4Statistical Analysis

The results obtained from the experiments were expressed as “mean value ± standard error” ($x \pm$ SEM). The significance between the groups was determined using one-way ANOVA test and followed by Fisher’s post-hoc LSD (least significant differences). All statistical procedures were performed by the “IBM SPSS Statistics Version 20” statistical program and $p<0.01$ was considered as significant.

3. Results

When living cells were counted by hemocytometer, the experimental groups decreased significantly with control, depending on the dose (Table 1). The average number of cells in the control and experimental groups was 48.4×103 (±1.6), 21.6×103 (±1.08), 17.4×103 (±1.12) and 11.4×103 (±0.79), respectively. Cells in the experimental groups were statistically significant compared to the control group compared to the decrease in the experimental groups ($p<0.05$).

Experimental groups (10 µg, 100 µg and 1,000 µg paclitaxel) were found to be statistically significant

Table 1 The effect of paclitaxel on *in vitro* MCF-7 cell proliferation.

Groups	*N*	Mean (±standard deviation)
Control	16	48.4±7.75
10 µg paclitaxel	16	21.6±4.28
100 µg paclitaxel	16	17.4±3.86
1,000 µg paclitaxel	16	11.4±4.12

Data were expressed as mean ± standard deviation.

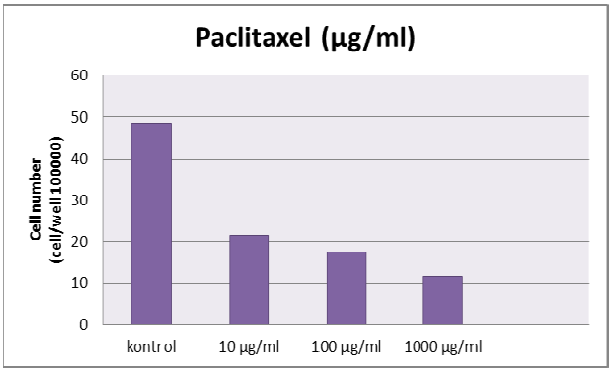

Fig. 1 Cell distribution between groups.

compared to each other, showing a decreased dose of cells due to high dose (p<0.05) (Figure 1).

In addition, the results of the regression analysis showed that cell viability decreased depending on the dose, that is, cell death increased as the dose increased.

4. Discussion

Cancer is defined as the uncontrolled growth of cells [8]. Breast and lung cancers are more common in women and prostate and lung cancers are more common in men. Advances in diagnosis and treatment in recent years have been reported to increase survival rate after diagnosis. In general, methods such as surgery, radiotherapy, chemotherapy and immunotherapy are used in cancer treatment [9,10].

Due to the leading cause of deadly diseases, there is a lot of research on cancer today. While some of these studies focus on cancer biology, some are done to improve the ways of treatment. Cell lines obtained from tumors developed *in vivo*, *in vitro* or spontaneously can also be used in studies on cancer. One of them is MCF-7. The most commonly used MCF-7 cell line in laboratory research worldwide is a weak aggressive and non-invasive cell line, which generally has low metastatic potential [11]. MCF-7 cells are universally used in research for ER (estrogen receptor) positive breast cancer cells. MCF7 cells have been reported to be compatible for anti-hormone therapy resistance studies because they are easily cultured and have ER expression when treated. MCF-7 cell populations modified to various anti-hormone environments were produced to investigate the characteristics of acquired anti-hormone resistant breast cancer cells. MCF-7 cell line provides practical information in breast cancer diseases [12].

Paclitaxel is considered a chemotherapeutic drug for the treatment of breast cancer. It has an apoptotic effect on high concentrations of cancer and consequently its treatment is associated with serious side effects, including gastrointestinal, pulmonary and neuromuscular toxicities, and neutropenia, granulocytopenia and hypotension [13,14].

Chemotherapy is a form of treatment that mainly aims at killing cancer cells. However, the effectiveness of existing chemotherapy agents in different types of cancer is limited. The cancer cells are the target cancer cells. An important process in treatment is achieved by stopping the death or proliferation of cancer cells. In our study, we wanted to evaluate the effects of these proteins in a tumor model by examining Netrin 1 expression and angiogenesis in Ehrlich solid tumor mass.

Some chemotherapy drugs such as paclitaxel can help control the disease by reducing vascularization of tumors [15].

Paclitaxel is also used as a water soluble taxol antineoplastic chemotherapeutic agent that stimulates apoptosis in breast, ovarian, prostate and cervical cancer cells [16,17].

When the control and experimental groups of our study were compared, it was observed that the treatment group induced apoptosis. Angiogenesis is

required for the proliferation and growth of cancer cells, and it has been reported that tumor tissues alsoinitiate vascular development [18,19]. Therefore, vascularization is prevented by using antiangiogenic substances as a way of cancer treatments [20,21]. In our study, it was observed that angiogenic activity decreased in the experimental groups where paclitaxel was applied in the control group, where angiogenic activity was high.

Studies have shown that paclitaxel exerts its antiangiogenic effect either by its metabolic effect in endothelial cells or by reducing its VEGF (vascular endothelial growth factor) levels [22-24]. Paclitaxel application has been reported to increase apoptosis in breast cancer cells [25].

In their studies on colorectal cancer, Pavlidis et al.[26] found that using bevacizumab chemotherapically restricts the growth of malignant cells and reduces angiogenesis.

In our study, the effect of paclitaxel on MCF-7 was investigated. Apoptosis can be measured using a variety of methods, utilizing the biochemical, morphological and molecular changes that occur in a cell during this process. Apoptotic and living cells were counted using MTS test method. The results of this study clearly demonstrated apoptotic events with a significant decrease in cell viability depending on the paclitaxel-administered MCF-7 cell line. These observations are compatible with other *in vitro* and *in vivo* studies showing that treatment of the cancer cell with paclitaxel causes induction of apoptosis. The apoptotic effect of paclitaxel suggests that it may be an alternative way to treat cancer diseases.

References

[1] Carmeliet,P.,and Jain, R.K. 2000.“Angiogenesis in Cancer and Other Diseases.”*Nature* 407: 249-57.

[2] Hanahan,D., and Folkman, J. 1996. “Patterns and Emerging Mechanisms of the Angiogenic Switch during Tumorigenesis.”*Cell*86: 353-64.

[3] Auerbach,R., Auerbach, W., and Auerbach, I. 1991.“Assays for Angiogenesis: A Review.”*Polakowski. Pharmacol. Ther*51:1-11.

[4] Scappaticci, F.A.2002.“Mechanisms and Future Directions for Angiogenesis-Based Cancer Therapies.”*J.Clin. Oncol* 20:3906-27.

[5] Sarkar, F.H., and Li, Y. 2002. “Mechanisms of Cancer Chemoprevention by Soy Isoflavone Genistein.”*CancerMetastasisRev* 21:265-80.

[6] Thaloor, D., Singh,A.K., Sidhu,G.S., Prasad,P.V., Kleinman, H.K., and Maheshwari, R.K. 1998.“Inhibition of Angiogenic Differentiation of Human Umbilical Vein Endothelial Cells by Curcumin.”*Cell GrowthDiffer* 9:305-12.

[7] Singh, A.K., Seth,P., Anthony,P., Husain, M.M., Madhavan, S., Mukhtar,H.,and Maheshwari, R.K.2002. “Green Tea Constituent Epigallocatechin-3-Gallate Inhibits Angiogenic Differentiation of Human Endothelial Cells.”*Arch. Biochem. Biophys* 401:29-37.

[8] Folkman, J. 1986.“How is Blood Vessel Growth Regulated in Normal and Neoplastic Tissue? G.H.A. Clowes Memorial Award Lecture.”*CancerRes*46: 467-73.

[9] Ogden, J. 1998. *Health Psychology: A Text Book*. Buckingham, UK: Open University Press.

[10] Jemal, A., Murray, T., Samuels, A., et al. 2003.“Cancer Statistics, 2003.”*Cancer J Clin* 53: 5-26.

[11] Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M.2001.“Cancer Statistics, 2001.”*Cancer J Clin* 51: 15-36.

[12] Gest, C., Joimel, U., Huang, L., Pritchard, L. L., Petit, A., Dulong, C., et al. 2013. “Rac3 Induces a Molecular Pathway Triggering Breast Cancer Cell Aggressiveness:Differences in MDA-MB-231 and MCF-7 BreastCancer Cell Lines.” *BMCCancer* 13: 63.

[13] Sweeney, E. E., Mcdaniel, R. E., Maximov, P. Y., Fan, P., and Craig, V. 2013.“Models and Mechanisms of Acquired Antihormone Resistance in Breast Cancer: Significant Clinical Progress despite Limitations.”*Horm Mol Biol Clin Investig* 9: 143163.

[14] Rowinsky, E. K., Eisenhauer, E. A., Chaudhry, V., Arbuck, S. G., and Donehower, R. C. 1993. “Clinical Toxicities Encountered with Paclitaxel.”*Semin Oncol*20: 1-15.

[15] Priyadarshini, K., and Keerthi, A. U.2012. “Paclitaxel against Cancer: A Short Review.”*Med Chem.*2 (7).

[16] Pamir, A. 2005. *Tıbbi Onkoloji Kitabı*. Ankara Üniversitesi Tıp Fakültesi Antıp Yayınları, 145.

[17] Singla, A. K., Garg, A., and Aggarwal, D. 2002.“Paclitaxel and Its Formulations.”*Int J Pharm*235: 179-92.

[18] Jordan, M. A.2002. “Mechanism of Action of Antitumor Drugs that Interact with Microtubules and Tubulin.” *CurrentMedicinal Chemistry-Anticancer Agents*2: 1-17.

[19] Kajdaniuk, D., Marek, B., Foltyn, W., and Kos-Kudła, B. 2011.“Vascular Endothelial Growth Factor (VEGF)-Part

1: In Physiology and Pathophysiology."*Endokrynol Pol* 62: 444-55.

[20] Kajdaniuk, D., Marek, B., Foltyn, W., and Kos-Kudła, B. 2011. "Vascular Endothelial Growth Factor (VEGF)-Part 2: In Endocrinology and Oncology."*Endokrynol Pol* 62: 456-64.

[21] Inoue, K., Slaton, J. W., and Karashima, T. 2000. "The Prognostic Value of Angiogenesis Factor Expression for Predicting Recurrence and Metastasis of Bladder Cancer after Neoadjuvant Chemotherapy and Radical Cystectomy."*Clin Cancer Res* 6: 4866-73.

[22] Chabannes, E., Bernardini, S., Wallerand, H., and Bittard, H. 2001. "Angiogenesis in Bladder: Prognosis Indicator and Therapeutic Target."*Prog Urol* 11: 417-27.

[23] Merchan, J. R., Jayaram, D. R., Supko, J. G.,et al. 2005. Increased Endothelial Uptake of Paclitaxel as a Potential Mechanism for Its Antiangiogenic Effects: Potentiation by Cox-2 Inhibition."*Int. J. Cancer* 113: 490-8.

[24] Loo, W. T. Y., Fong, J. H. M., and Chow, L. W. C. 2005. "The Efficacy of Paclitaxel on Solidtumour Analysed by ATP Bioluminescence Assay and VEGF Expression: Atranslational Research Study." *Biomedicine&Pharmacotherapy* 59: 337-9.

[25] Pasquier, E., Honore, S., Pourroy, B., Jordan, M. A., Lehmann, M., Briand, C., et al. 2005. "Antiangiogenic Concentrations of Paclitaxel Induce an Increase in Microtubule Dynamics in Endothelial Cells but Not in Cancer Cells."*Cancer Res* 65:2433-40.

[26] Pavlidis, E. T., and Pavlidis, T. E. 2013. "Role of Bevacizumab in Colorectal Cancer Growth and Its Adverse Effects: A Review."*World J Gastroenterol* 19 (31):5051-60.