The Cytotoxicity of Paclitaxel Was Smaller than Doxorubicin in T47D Breast Cancer Cell

Irma Yanti Rangkuti
Biomedic/Pharmacology Department, Faculty of Medicine, Universitas Islam Sumatera Utara, Indonesia

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Abstract: Breast cancer is a disease in which there is excessive growth or uncontrolled development of breast tissue cells. Breast cancer is a type of cancer commonly found by most women, ranking fifth leading cause of cancer worldwide cancer, consist of various subtypes. Chemotherapy is one of therapy in breast cancer patients but the benefit is not equal for all patients and paclitaxel is one of the most important chemotherapeutic drugs. The descriptive study had been done to paclitaxel and doxorubicin in T47D breast cancer cell. This study aimed to compare cytotoxic effect of paclitaxel and doxorubicin in T47D breast cancer cell, used paclitaxel concentrations 1000; 500; 250; 62,5; 31,25; and 15,625 nM and doxorubicin concentrations 500; 250; 125; 62,5; 31,25; and 15,625 nM for 24 hours. The study was an in vitro. The cytotoxic test used MTT method to determine IC50 and analyzed by SPSS. The result showed that IC50 paclitaxel was $1577.2 \pm 115.3$ nM and IC50 doxorubicin $202.37 \pm 3.99$ nM. The cytotoxicity of paclitaxel was smaller than doxorubicin in T47D breast cancer cell.

1 INTRODUCTION

Breast cancer is a disease in which there is excessive growth or uncontrolled development of breast tissue cells. Breast cancer is a type of cancer commonly found by most women, ranking fifth leading cause of cancer worldwide cancer around 522,000 deaths and the most common cause of death in women in developing countries (324,000 deaths). According to IARC's Globocan data (International Agency for Research on Cancer) in 2012 there are 14,067,894 new cases of cancer and 8,201,575 deaths from cancer worldwide with the most cancer types of breast cancer, prostate cancer and lung cancer. Estimated 1.67 million new cases breast cancer in 2012. For Indonesia, the incidence of cancer in women is about 134 per 100,000 population with most cases of breast cancer of 40 per 100,000 women. Globocan estimates, deaths in Indonesia due to breast cancer approximately 16.6 deaths per 100,000 population (Kemenkes RI, 2016).

Breast cancer consist of subtypes based on IHC markers including ER, progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Breast tumors are grouped into four basic subgroups according to these markers, i.e., [ER+|PR+]HER2 - (tumors with either ER or PR positivity, and HER2 negativity), [ER+|PR+]HER2+ (tumors with either ER or PR positivity, and HER2 positivity), ER-PR-HER2+ (tumors with ER and PR negativity, and HER2 positivity, also named HER2 positive), ER-PR-HER2- (tumors with ER, PR, HER2 negativity, also named triple negative) (Dai et al. 2016).

Chemotherapy is one of therapy in breast cancer patients and improves survival of patients with stage I–III breast cancer, but the benefit is not equal for all patients because there are molecular characteristics of the cancer affect sensitivity to chemotherapy (Andre and Pusztai 2006; Hassan et al. 2010). The use of cytotoxic chemotherapy in both advanced and early stage breast cancer has made significant progress in the last 10 years with several landmark studies identifying clear survival benefits for newer therapies (Hassan et al. 2010).

Paclitaxel is one of the most important chemotherapeutic drug, isolated from the Pacific yew, Taxus brevifolia, was approved for the treatment of metastatic breast cancer in 1994 (Patt, Gauthier, and Giordano 2006). This drug causes abnormal stabilization of the dynamic microtubule polymerization that alters intracellular signaling, transport organelle and locomotion, and leading to the failure of mitosis (Honore, Pasquier, and Braguer 2005). Recent studies showed that paclitaxel is able to induce reactive oxygen species (ROS) production in cancer cells and hydrogen peroxide (H2O2) that

Doxorubicin is considered to be the most effective agent in the treatment of breast cancer patients (Smith et al. 2006). Doxorubicin is an anthracyline drug first extracted from Streptomyces peucetius var. caesius in the 1970’s. The mechanisms of action doxorubicin in cell are intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and generation of free radicals and their damage to cellular membranes, DNA and proteins. Doxorubicin is oxidized to semiquinone, an unstable metabolite, which is converted back to doxorubicin in a process that releases reactive oxygen species. Reactive oxygen species can lead to lipid peroxidation and membrane damage, DNA damage, oxidative stress, and triggers apoptotic pathways of cell death (Thorn et al. 2011).

The T47D cell is one of breast cancer cell that used for research. The characteristic this cell is a continuous cell line isolated from a breast ductal tumor tissue of a 54-year-old woman who expresses a mutated p53 protein (missense mutation) at a 194 residue (in zinc-binding domain, L2).

2 MATERIALS AND METHODS

This study used paclitaxel and doxorubicin as materials. Paclitaxel was obtained from PT Dankos Farma, Indonesia.

This research was an invitro, descriptive study to compare cytotoxic effect of paclitaxel and doxorubicin in T47D breast cancer cell, used paclitaxel concentrations 1000; 500; 250; 31,25; 15,625 nM and doxorubicin concentrations 500; 250; 62,5; 31,25; and 15,625 nM for 24 hours. The cytotoxic test used MTT [3-(4,5-dimetiltiazol-2-il)-2,5-difenil tetrazolium bromida] method to determine IC50 and analized by SPSS.

2.1 Cell Culture

In this study, T47D cells obtained from the Laboratory of Parasitology, Faculty of Medicine, Gadjah Mada University were grown in RPMI medium containing 10% Fetal Bovine Serum (Gibco, USA), 2% Penicillin-Streptomycin (Gibco, USA), and Fungizone (Amphotericin B) 0.5% (Gibco, USA) on the flask in a humidified incubator(5% CO2/95% air) at 37°C (Doyle and Bryan, 1998).

2.2 Cell Viability Assay

The viability of T47D cells was assessed using the MTT assay. The cells were cultivated on 96 well plates (Iwaki, Japan). Each well contains 1x10^4 cells. The cells incubated in a humidified incubator (5% CO2/95% air) for 24 hours. After 24 hours incubation, the culture medium is discharged and each well is given paclitaxel with concentration 1000; 500; 250; 31,25; and 15,625 nM. After 4 hours incubation, the cells was incubated with 0.5 mg/mL MTT (Sigma-Aldrich, USA) for 4 hours at 37°C. Then, the cells are incubated for 24 hours at room temperature and protected from light. After incubation, cells were shaken, and cell absorbance was measured by elisamicroplate reader (Bio-Rad, USA) at λ 595 nm. The experimental data were the absorbance of each well, and then converted to percentage of a cells viable using equation as indicated below

\[
\text{% of viable cells} = \frac{B - C}{A - C} \times 100\% \quad (1)
\]

Where A, B, and C (1) respectively are absorbance of control cells absorbance, treated cells absorbance, and medium culture absorbance. All data were expressed as IC50 that calculate using probate regression analysis at SPSS, test were used for statistical analyses with p values <0.05 were considered significant (Meiyanto et al. 2008).

3 RESULT AND DISCUSSION

Cytotoxic assay is a preliminary test to determine the potential toxicity of a compound and IC50 as a mainly parameters. T47D cells were exposed to paclitaxel using concentration series of 1000; 500; 250; 31,25; and 15,625 nM for 24 hours. After analyzed, IC50 paclitaxel = 1577,2 ± 115,3 nM.

The result of cytotoxic test paclitaxel against T47D cells during 24 hours exposure can be seen in Figure 1.
In the cytotoxic paclitaxel test, doxorubicin was used as a positive control, one of the chemotherapy for breast cancer using concentration series of 500; 250; 62.5; 31.25 and 15.625 nM. After analysis, IC$_{50}$ doxorubicin = 202.37 ± 3.99 nM. The result of doxorubicin cytotoxic test can be seen in Figure 2 below.

![Graph of doxorubicin concentration vs viability](image)

**Figure 2:** Graph the effect of doxorubicin concentration on viability T47D cell.

This study used the MTT assay to test cytotoxicity of drug, a quantitative method was measured by *elisamicroplate* reader (Bio-Rad, USA) at $\lambda$ 595 nm. Cytotoxic effects are indicated by IC$_{50}$ values, the concentration that causes death in 50% of the cell population by calculating living cells. The principle of MTT assay is colorimetry (measurement of color intensity) based on the formation of formazan crystals (purple and filamentous) in living cells, penetrating the membrane and accumulating in them (Figure 3 - 8). Color formation in living cells as a result of metabolizing a substrate by living cells into colored products. The tetrazolium succinate reductase system found in the living cell mitochondria included in the respiratory chain will reduce the MTT yellow to form purple formazan crystals (van Meerloo, Kaspers, and Cloos, 2011).

Dead cells cannot form formazan crystals because dead cells are unable to aspirate so that tetrazolium succinate enzymes which can reduce MTT salt to formazan products are not produced, so the color of dead cells is not purple but will remain yellow. The more cells that live, the purple will become thicker (Freshney, 2015). These figures below were T47D breast cancer cell looked by microscope.

**A. T47D breast cancer cell before MTT**

These figures below showed T47D cell before MTT, consist of control cell, T47D with doxorubicin exposure and paclitaxel (Figure 3 – 5).

![Control cell (T47D without drug)](image)

**Figure 3:** Control cell (T47D without drug).

![T47D cell after giving doxorubicin 500 nM for 24 hours.](image)

**Figure 4:** T47D cell after giving doxorubicin 500 nM for 24 hours.
Based on IC_{50} from cytotoxic test, it showed that cytotoxicity of paclitaxel was smaller than doxorubicin. Study in 2012 found that paclitaxel was resistant in T47D breast cancer cell associated with highly expresses Lin28 in T47D cells than the MCF7, Beap-37 or SK-BR-3 cancer cell lines, which had low-level expression of Lin28. This study knocked down of Lin28 in Lin28 high expression T47D cells, the result showed increasing the sensitivity to paclitaxel treatment, while stable expression of Lin28 in breast cancer cells effectively attenuated the sensitivity to paclitaxel treatment, resulting in a significant increase of IC_{50} values of paclitaxel. Transfection with Lin28 also significantly inhibited paclitaxel-induced apoptosis. This study showed that Lin28 expression was dramatically increased in tumor tissues after neoadjuvant chemotherapy or in local relapse or metastatic breast cancer tissues. Moreover, further studies showed that p21, Rb and Let-7 miRNA were the molecular targets of Lin28. Overexpression of Lin28 in breast cancer cells considerably induced p21 and Rb expression and inhibited Let-7 miRNA levels (Lv et al. 2012).

p21, universal inhibitor of cyclin kinases inhibits the activity of each member of the cyclin/CDK family (Xiong et al. 1993), promote cell cycle arrest in cell cycle (Karimian, Ahmadi, and Yousefi, 2016). Cell cycle is a cell proliferation process that mediates the growth and development of living things (Nurse, 2000). The Rb protein is a tumor suppressor, which plays a pivotal role in the negative control of the cell cycle and in tumor progression. It
has been shown that Rb protein (pRb) is responsible for a major G1 checkpoint, blocking S-phase entry and cell growth (Giacinti and Giordano, 2006; Foster et al. 2001).

Doxorubicin is an anthracycline breast cancer drug that is still used in combination regimens and also for other types of cancer such as leukemia (Wattanapatayakul et al. 2005). Doxorubicin was used as a positive control in this study, because this drug is still used, but also doxorubicin showed an anticancer effect on T47D cells (Barzegar et al. 2015). Cytotoxic activity of doxorubicin through topoisomerase II inhibition, DNA intercalation, cell membrane binding and semiquinone free radical formation and oxygen free radicals (Bruton et al. 2005). Doxorubicin causes the activation of various molecular signals from AMPK (AMP-activated protein kinase inducing apoptosis) to influence the Bcl-2/Bax apoptosis pathway. By altering the Bcl-2/Bax ratio, downstream activation of different caspases can occur resulting in apoptosis (Tacar, Srimornsak, and Dass, 2013)

4 CONCLUSION

IC₅₀ paclitaxel was 1577.2 ± 115.3 nM and IC₅₀ doxorubicin 202.37 ± 3.99 nM. The cytotoxicity of paclitaxel was smaller than doxorubicin in T47D breast cancer cell.

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