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The Cytotoxic Test of Metformin Hydrochloride to T47D Breast Cancer Cell

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ABSTRACT

Breast cancer is a disease in which there is excessive growth or uncontrolled development of breast tissue cells. Cancer is a disease caused by genetic disorders caused by DNA mutations that cause loss of growth control. This genetic disorder affects the cell cycle and cell apoptosis and causes the formation of cancer and decreased therapeutic response to cancer drugs. Metformin is an antihyperglycemic in type 2 diabetes mellitus patient. The decrease in cancer risk occurs in patients with type 2 diabetes mellitus who use metformin. The cytotoxic metformin test was performed in an in vitro to T47D cells using paclitaxel as a positive control. The concentration of metformin HCl used was 5000; 2500; 1250; 312.5; and 156.25 µM. The concentration of paclitaxel used is 1000; 500; 250; 31.25 and 15.625 nM. Cytotoxic test using MTT method to determine IC_{50}. Data were analyzed using probit analysis using SPSS 22 version. The result of the cytotoxic test showed that IC50 metformin HCl was 13457.3 ± 1096,5µM. While IC50 paclitaxel as control is 1577.2 ± 115.3 nM.

INTRODUCTION

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. Among women, breast cancer is most prevalent mainly in developing countries with an estimated 1.67 million new cases in 2012 (Kemenkes RI, 2016).

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). 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).

TP53 is the most commonly mutated gene in human cancer and for breast cancer the p53 mutation occurs in approximately 35% of cases that usually occur in ductal carcinoma, aggressive histopathologic tumors such as large tumor size (> 20 mm), positive lymph nodes, estrogen receptor (ER) and negative progesterone receptor (PR), high-level anaplasia and high proliferation rates (Lacroix *et al.*, 2006), so breast cancer patients with p53 mutations had poorer survival rates than patients without p53 mutations (Gasco and Crook, 2003; Olivier *et al*., 2006). For patients with breast cancer with p53 mutations tend to experience relapse than patients without mutations when given anthracycline-based monotherapy (Aas *et al.*, 1996). Other studies have also mentioned that breast cancer patients with p53 mutations have a poorer response to doxorubicin monotherapy(Geisler et al., 2003; Di Leo *et al.*, 2007). Therapeutic resistance is not only to anthracycline groups but also to non-anthracyclin groups, CMF (Andersson *et al.*, 2005) and also to 5-fluorouracil and mitomycin C (Geisler et al., 2003).

Metformin, biguanide group is the drug of choice for type 2 diabetes mellitus patients. Anti-cancer effects of metformin based on studies of a reduced risk of cancer occurring in patients with type 2 diabetes mellitus who used metformin (Lin *et al.*, 2015; Francosi*et al.*, 2013; Gandini, *et al.*, 2014).

The T47D 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 the zinc-binding domain, L2). Because p53 mutations cause resistance to chemotherapy in breast cancer, the researcher was interested in conducting metformin effect studies on the viability of T47D cancer cells.

MATERIALS AND METHODS

a. *Metformin hydrochloride*

Metformin hydrochloride is obtained from PT. Dexa Medica Palembang Indonesia.

b. Cell Culture

In this study, T47D cells obtained from the Laboratory of Parasitology, Faculty of Medicine, GadjahMada 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% CO_2/95% air) at 37°C(A Doyle and Bryan, 1998).

c. 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 were incubated in a humidified incubator (5% CO_2/95% air) for 24 hours. After 24 hours incubation, the medium culture is discharged and each well is given metformin with concentration 5000; 2500; 1250; 312,5; 156,25 µM. After 24 hours incubation, the cells were incubated with 0.5 mg/mL MTT (Sigma-Aldrich, USA) for 4 hours at 37°C. The cells that are feasible to react with MTT to produce of purple crystals formazan. After 4 hours, 10% SDS (Sigma-Aldrich, USA) stopper in 0.01 N HCl (Merck, USA) was added to dissolve the formazan crystals. Then, the cells are incubated for 24 hours at room temperature and protected from light. After incubation, the cells were shaken, and cell absorbance was measured by ELISA microplate reader (Bio-Rad, USA) at λ 595 nm. The experimental data were the absorbance of each well, and then converted to a percentage of cells viable using equation as indicated below

$$\%\ of viable cells = \frac{B-C}{A-C} x\ 100\% \qquad (1)$$

Where A, B, and C (1) respectively are the absorbance of control cells absorbance, treated

cells absorbance, and medium culture absorbance. All data were expressed as IC_{50} that calculate using probit regression analysis at SPSS 22, a test was used for statistical analyses with p values < 0.05 were considered significant (Meiyanto, *et al*, 2008).

RESULT AND DISCUSSION

The cytotoxic assay is a preliminary test to determine the potential toxicity of a compound and IC_{50} as the main parameter.

The result of cytotoxic metformin test against T47D cells during 24 hours exposure can be seen in Figure 1

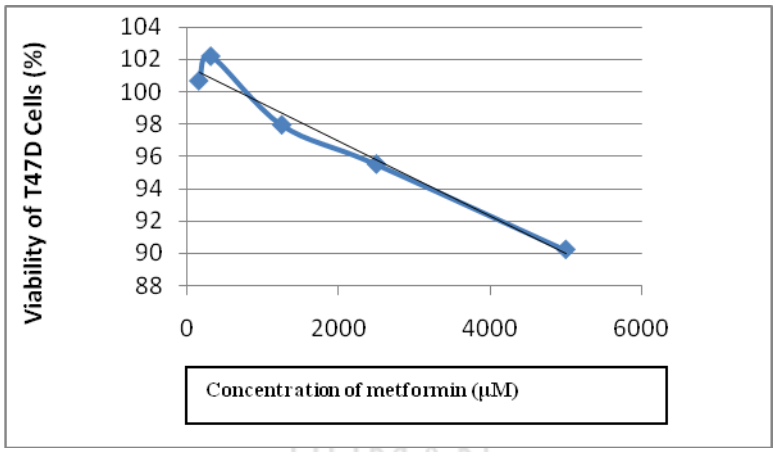

Figure 1. Graph the effect of metformin concentration on T47D cell viability

T47D cells were exposed to metformin using concentration series of 5000; 2500; 1250; 312.5; 156.25 µM for 24 hours. After analyzed, IC_{50} value obtained = 13457.3 ± 1096,5µM.

In the cytotoxic metformin test, paclitaxel was used as a positive control, one of the chemotherapies for breast cancer using a concentration series of 1000; 500; 250; 31.25 and 15.625 nM. After analysis, IC_{50} paclitaxel = 1577.2 ± 115.3 nM. The result of Paclitaxel cytotoxic test can be seen in Figure 1.2 below.

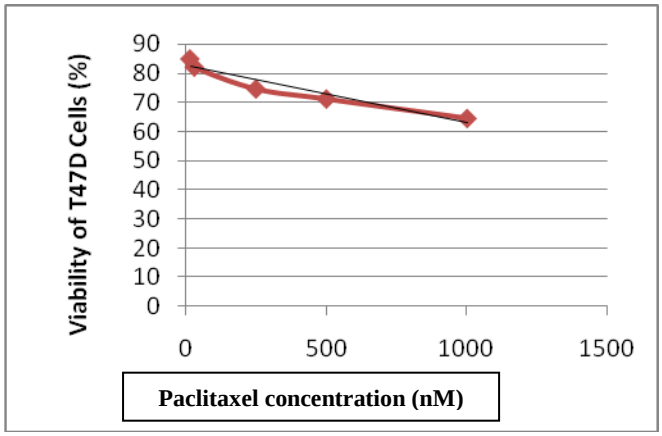

Figure 2. Graph the effect of paclitaxel concentration on viability T47D cell.

From the cytotoxic test found metformin could inhibit the growth of T47D cancer cells with a greater concentration than paclitaxel. Metformin inhibited the complexes of the respiratory chain I in mitochondria (Birsoy*et al*, 2014; Owen *et al*, 2000; Wheaton, *et al*, 2014). The complex constraint I in the respiratory chain of mitochondria leads to a decreased cell concentration of ATP and increasing AMP will further stimulate AMP-Kinase (Queiroz *et al*, 2014). Adenosine monophosphate-activated protein kinase (AMPK) was a regulator of low cellular response in all eukaryotic cells. AMPK is activated when intracellular concentration of adenosine triphosphate (ATP) decreased and the concentration of adenosine monophosphate (AMP) increased (Zhou, *et al* 2001). The activated AMP-Kinase phosphorylates various substrates such as ACC, HMG-CoA reductase, PFKFB2, TBC1D1, TSC2, Raptor, TIF-1A, p-53, etc. which caused various effects such as activating the catabolic pathway (glycolysis) and inhibiting the pathway anabolic deficiency inhibition of fatty acid synthesis, cholesterol, ribosomal RNA synthesis, resistance to cell cycle through AMPK phosphorylation top-53 causing an increased in p-21 transcription of a CDK (cyclin dependent kinase) inhibitor that plays a role in the cell cycle, and had an effect on apoptosis (Hardie, 2011).

Metformin effect as an anti-tumor occurred through AMPK / LKB1 / TORC 1 mechanism that caused apoptosis in cancer cells. TORC1 resistance occurred due to AMPK activation of its substrate (TSC2 and raptor) (Kim, *et al*, 2004; Koo, *et al*., 2005; Shaw, *et al*., 2004; Woods, *et al*., 2003).

Previous *in vitro*studies have been tested for several types of cancer cells showing the emphasis on the growth and apoptosis of cancer cells such as WiDr cells for colorectal cancer

cells (Wibowo, *et al*., 2015), Hep G-2 cells for liver cancer cells (Cai, et al., 2013), and MCF-7 cells (Queiroz *et al.*, 2014), MDA-MB-231 (Li P Zhao, et al., 2015), MDA-MB-435 for breast cancer cells.

The metformin study of T47D cells suggested that metformin inhibited T47D cell growth through AMPK activation and histo H2B monophybitinizing inhibition (Du Y, et al., 2014) and metformin causing apoptosis of T47D cells and enhancing the expression of caspases 8 and 9 (Haji H, *et al*., 2016).

CONCLUSION

Metformin had a cytotoxic activity on T47D cells with IC_{50} = 13457.3 ± 1096,5 μM.

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