EFFECT OF CISPLATIN ON TRIPLE NEGATIVE BREAST CANCER CELLS

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Specially dedicated to my family

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ABSTRACT

Triple negative breast cancer is a distinct group of breast tumors that is characterized by the lack of expression of hormone receptors including estrogen (ER) and progesterone (PgR) receptors as well as human epidermal growth factor receptor 2 (HER2). This subtype is a high risk breast cancer with the existence of breast cancer stem cells (BCSCs) which is presumed to be more aggressive and difficult to cure. In this *in vitro* study, the effect of cisplatin as a member of the platinum anticancer drugs in two different breast cancer cell lines, that represent the triple negative (MDA-MB-468) and hormone positive Luminal A (MCF-7) breast cancer subtypes were examined. Cisplatin was used at 5 different concentrations 5, 10, 15, 20 and 25µM, and cell viability was measured using celltiter glo assay while proliferation was measured using the cyquant NF Cell Proliferation Assay. Apoptosis was analyzed via caspase glo R 3/7 assay. The cell viability for MDA-MB-468 cell line started to decline to 85% at dose 20µM of cisplatin and 75% at 25µM of cisplatin while viability in MCF-7 cell lines was reduced to 91% in the highest dose. Cisplatin showed anti proliferation effect for both cell lines started from the lowest concentration of the drug 5µM in MDA-MB-468 cell lines and at concentration of 15µM for MCF-7 cell line. MDA-MB-468 showed changes in cell morphology at the dose of 25µM representing that small numbers of cells were damaged and disturbed in cell shape while MCF-7 showed no changes in cell morphology under the light microscope. Cisplatin at 25µM did not induce apoptosis in MDA-MB-468 cell line in contrast with the control drug taxol at 50nM which induced apoptosis in MDA-MB-468 cell line. It can be concluded that cisplatin inhibits cell growth in a dose dependent manner and subsequent cytotoxicity resulting in morphological changes of the cancer cells, obvious reduction in cell viability and proliferation. Based on the findings, it can be concluded that cisplatin may induce differentiation in TNBCs. However, more investigations are needed to confirm its ability to induce differentiation of breast cancer cells.

ABSTRAK

Kanser payudara tiga ganda negatif (TNBC) adalah kumpulan khas tumor payu dara yang dicirikan oleh kekurangan mengekspresikan reseptor hormone termasuk estrogen (ER) dan progesteron (PGR) serta reseptor 2 faktor pertumbuhan epidermis manusia (HER2). Kanser payudara subjenis ini adalah berisiko tinggi dengan kewujudan sel-sel stem kanser payu dara (BCSCs) yang dianggap menjadikannya lebih agresif dan sukar untuk diubati. Dalam kajian *in vitro* ini, kesan cisplatin sebagai salah satu ubat antikanser platinum dalam dua bahagian sel kanser payu dara yang berbeza, yang mewakili tiga ganda negatif (MDA-MB-468) dan hormon positif Luminal A (MCF-7) subjenis kanser payu dara telah diperiksa. Cisplatin telah digunakan dalam lima kepekatan yang berbeza iaitu 5, 10, 15, 20 dan 25μM, dan daya maju sel diukur dengan menggunakan 'celltiter glo assay' manakala percambahan diukur dengan menggunakan 'cyquant NF Cell Proliferation Assay'. Apoptosis dianalisis melalui 'caspaseglo R 3/7assay'. Dayamaju sel bagi bahagian sel MDA-MB-468 mula merosot kepada 85% pada dos 20μM cisplatin dan 75% pada dos 25μM cisplatin, manakala dayamaju sel bagi bahagian sel MCF-7 telah merosot kepada 91% pada dos ubat yang tertinggi. Cisplatin menunjukkan kesan anti percambahan untuk kedua-dua bahagian sel bermula dari kepekatan ubat yang paling rendah, 5mM untuk bahagian sel MDA-MB-468 dan 15μM untuk bahagian sel MCF-7. Cisplatin pada kepekatan 25μM tidak mendorong apoptosis pada kedua-dua bahagian sel, berbeza dengan taxol pada 50μM yang mendorong apoptosis dalam bahagian sel MDA-MB-468. Dapat disimpulkan bahawa cisplatin menghalang pertumbuhan sel dengan cara yang bergantung kepada dos dan cytotoxicity seterusnya menyebabkan perubahan morfologi kepada sel-sel kanser, pengurangan ketara dalam dayamaju dan percambahan sel. Berdasarkan penemuan ini, dapat disimpulkan bahawa cisplatin boleh menyebabkan pembezaan dalam TNBCs. Walaubagaimanapun, siasatan yang lebih lanjut diperlukan untuk mengesahkan keupayaan cisplatin mendorong pembezaan pada sel-sel kanser payu dara.

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LIST OF ABBREVIATIONS

°C	-	degree Celsius (centigrade)
µl	-	microliter
µM	-	Micromole
ATCC	-	American Type Culture Collection
BCSCs	-	Breast cancer stem cells
BER	-	Base-excision repair
cDDP	-	cis diammine dichloride platinum
CKs	-	Cytokeratins
CO_2	-	Carbon dioxide
DMEM/F12	-	Dulbecco's Modified Eagle Medium/Nutrient Mixture
DNA	-	Deoxyribo nucleic acid
EMT	-	Epidermal mesenchymal transition
ER	-	Estrogen hormonal
FBS	-	Fetal Bovine Serum
FDA	-	Food and Drug Administration
HBSS	-	Hanks' Balanced Salt Solution
HER2	-	Human epidermal growth factor2 receptor
IHC	-	Immunohistochemistry
MCF-7	-	Michigan Cancer Foundation-7
Mg	-	milligram
miRNAs	-	MicroRNAs
Ml	-	millilitre
NER	-	Nucleotide-excision repair
nM	-	Nanomol
NT	-	non treated
PARP	-	Poly adenosine diphosphate–ribose polymerase
PBS	-	Phosphate Buffer Saline

PCR	-	polymerase chain reaction
PR	-	Progestron receptors
SEM	-	Standard Error of the Mean
TN	-	Triple negative
TNBC	-	Triple negative breast cancers

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Breast cancer is a malignant tumor that begins in the cells of the breast (Aktas *et al.,* 2009). A malignant tumor is a cluster of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body (Sobin *et al.,* 2011). Breast cancer is the main common diagnosed cancer in female both in the developed and less developed countries. Accounting for 23% of the overall new cancer patients and 14% worldwide, over 508 000 women died in 2011 due to breast cancer (Eittah *et al.,* 2014). Death rates for breast cancer have steadily decreased in women since 1989, with larger decreases in younger than in older women. The decrease in breast cancer fatality rates represents improvements in both early diagnosis and treatment (Siegel *et al.*, 2015).

Triple negative breast cancers (TNBC) are characterized as aggressive cancer with early visceral metastasis and subsequently poorer prognosis (Fan *et al.*, 2012). Most deaths occur in the first 5 years (Chacón and Costanzo, 2010). TNBCs lack important hormonal receptors (estrogen ER, progesterone and human epidermal growth factor2 receptor HER2) which are generally used as target therapy in these breast cancer subtype (Hastak *et al.*, 2010). Chemotherapy remains to be the simply probable therapeutic choice in the adjuvant or metastatic setting in TNBCs (Dangi

and Firodiya, 2012). Several efforts are recently being undertaken to develop treatment for patients with triple negative tumor as there are no known targeted therapies. Many therapeutics are used with different target agents including anti-angiogenesis (drugs stop tumors from making new blood vessels), proliferation signaling targeting, DNA binding agent and DNA repair mechanisms which significantly affect the response to cytotoxic treatments (Crown *et al.*, 2012; Dangi and Firodiya, 2012; O'shaughnessy *et al.*, 2011).

These tumors are known to contain high number of cancer stem cells (CSCs), which are difficult to be killed by chemotherapy, thus many attempts are focusing on the identification of targeted treatments that make this CSC population within the tumors more susceptible to chemotherapy (Hastak *et al.*, 2010; Tiwary *et al.*, 2011). Cisplatin chemotherapy regimens have shown promising anticancer affects in the treatment of difficult to treat cancers such as testicular, ovarian, cervical, gastric as well as small cell lung cancer (Bertolini *et al.*, 2009; Dasari and Tchounwou, 2014; Koizumi *et al.*, 2008). Cisplatin has multiple mechanisms of action, the primary mechanism involves, interaction with DNA to form DNA adducts and which activate a number of signal transduction pathways. Signal pathway including those involving ATR, p53, p73, MAPK and culminate in the activation of apoptosis (Fuertes *et al.*, 2003; Galluzzi *et al.*, 2012).

The platinum atom in cisplatin is known to form a divalent bond with the purine bases at N7, whereby the 1, 2 or 1, 3 intrastrand crosslinks take place and disrupt the DNA structure. This intrastrand crosslink between two adjacent G residues has shown to be the major damage resulting causing cytotoxicity (Basu and Krishnamurthy, 2010; O'Brien and Brown, 2006). The Cisplatin DNA adducts interfere with DNA replication and transcription but the mechanism of action still vague (O'Brien and Brown, 2006; Basu and Krishnamurthy, 2010). This research will focus on examining the anticancer effects of cisplatin on TNBC cells, by studying the effect of this drug on cell viability, proliferation and it's ability to induce apoptosis.

1.2 Problem Statement

Among all the breast cancer subtypes, TNBCs accounts for approximately fifteen percent of all types of breast cancers (Cleator *et al.*, 2007). It is associated with a worse prognosis due to lack of hormonal receptors (estrogen, progesterone and HER2) which leads to the ineffectiveness of hormonal therapies, leaving the chemotherapy as the only and most suitable treatment. In addition, patients with TNBC have tumor recurrence and the majority of deaths occur in the first 3 and 5 years following the primary treatment, respectively making the TNBC an invasive form of breast cancer which needs attention (Ovcaricek *et al.*, 2011). Although the TNBCs are specifically sensitive to cisplatin, the mechanism in which cisplatin induces anti-cancer effects in TNBCs are still unclear.

1.3 Objectives of the Study

Followings are the objectives proposed for this study:

i. To determine the effects of cisplatin on the growth and morphology of the triple negative breast cancer cells.

ii. To study the effect of cisplatin in inducing apoptosis in triple negative breast cancer cells.

1.4 Scope of Study

In vitro anticancer studies at the cellular level including cell viability, proliferation and apoptosis assays were conducted in two human breast cancer cell lines using cisplatin (cDDP).

1.5 Significance of the Study

This research focuses on the mechanism of action of cisplatin in TNBCs includes their effect on programmed cell death, as long as their cytotoxicity effects representing in cell viability and proliferation. Furthermore, it assists matching patient with the target therapy and contributes to reduce recurrency rate, surgery (mastectomy) and lowering the number of fatality, since TNBCs is one of leading cause of death on the world.

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