Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.02.Art026

Analysis of Carboplatin and STAT3 in the Breast Cancer MCF7 Cell Line

Manya Nautiyal[1], Dhanraj Ganapathy[2], Ameya K P[3], Durairaj Sekar[3*]
[1]*Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India*
[2]*Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical And Technical Science (SIMATS), Saveetha University, Chennai, India*
[3]*RNA Biology Lab, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, Chennai, India*

Abstract

Globally, the most common cancer to be diagnosed in women is breast cancer, surpassing lung cancer. One of the treatments used is carboplatin, which is a platinum-based drug. These substances function by preventing the growth of cancerous cells. We conducted a study to evaluate the dosage dependency of carboplatin in light of the adverse effects that have been recorded in patients. After being cultivated, the MTT test was used to determine the vitality of the MCF-7 breast cancer cell line. Afterwards, cDNA synthesis was carried out after RNA isolation using the TRIzol technique. Finally, a quantitative real-time polymerase chain reaction was used to analyze gene expression. Every single result was statistically examined using SPSS. The results demonstrated that MCF-7 cell lines multiplied both before and following carboplatin therapy. Furthermore, they showed that the STAT3 gene was expressed in MCF-7 both before and during the carboplatin treatment. According to our research, if carboplatin is taken at the lowest risk dosage, it can be very beneficial in treating breast cancer. However, since this is just an early study, more investigation and pre-clinical approval are required.

Keywords: *Breast Cancer, Carboplatin, Gene Expression, MCF-7, STAT3.*

Introduction

Despite ongoing medical progress, breast cancer remains the second most common and dangerous disease among women. Over the past 40 years, there has been a concerning rise in the incidence of breast cancer. Globally, there is expected to be 2.3 million new instances of breast cancer in 2020, and 6,85,000 deaths from the illness. There were notable geographical differences found between various nations and areas. It's noteworthy to notice that high-income nations account for a larger percentage of breast cancer mortality [1]. More than 3 million additional instances of breast cancer and more than 1 million deaths from the illness are predicted by 2040 [2]. This is likewise the situation in India, where 179,790 cases of breast cancer were reported in 2020. The primary reasons for the rising incidence of breast cancer include longer lifespans, lifestyle modifications (such as obesity, inactivity, tobacco use, and alcohol consumption), and reproductive health practices (such as parity, breastfeeding, oral contraceptive usage, and hormone replacement therapy). A further important risk factor is genetic predisposition. An increasing amount of research has discovered genetic variants associated with moderate and low risk of breast cancer. Women with mutations in either the BRCA1 or BRCA2 gene have a lifetime risk of breast cancer ranging from 65% to 72%. To use the risk management strategies and reduce the length of illness, increasing knowledge is crucial [3].

Received: 31.05.2024 ***Accepted: 04.06.2024*** ***Published on: 28.06.2024***
****Corresponding Author: duraimku@gmail.com***

The term "breast cancer" refers to the part of the body where the disease first appeared. It is defined by abnormal cell growth and division that starts in the breast tissue. Genetics, hormones, food, lifestyle, and environmental factors are among the many factors that can cause breast cancer [4]. The conventional approach to treating breast cancer involves using nanotechnology in addition to surgery, chemotherapy, and radiation treatment. However, there is some hope thanks to the new methods for treating breast cancer. These include gene therapy [5], oncogenes inactivation [6], tumour suppressor gene augmentation [7], cell target suicide [8], chemoprotective strategy [9], virus-mediated oncolysis [10], and immunomodulation [11]. There are now several research looking into natural sources as a possibly safer alternative.

In collaboration with Bristol-Myers, a cancer research organisation, and the Royal Marsden Hospital, Johnson Matthey first created carboplatin, also referred to as 1,1-cyclobutyl dicarboxylate. Professor Barnett Rosenberg found that carboplatin had chemo-preventive properties at the beginning of the 1960s. The primary distinctions in its pharmacological mechanism are related to its toxicity and molecular composition. For patients with gynaecological malignancies, combined therapy using platinum and taxanes as adjuvant and neoadjuvant therapies has been demonstrated to be helpful.

Furthermore, a lot of individuals with recurring gynaecological malignancies are given carboplatin again. Despite risks from recurrence, prior studies have shown that carboplatin therapy may have increased patients' overall survival [12]. Carboplatin is a platinum anticancer medicine used to treat a variety of human cancers. Compared to its first-generation equivalent, cisplatin, carboplatin has a structural feature that considerably limits its chemical reactivity [13]. This is due to its bidentate dicarboxylate chelate leaving ligand. APR Factor (APRF), often referred to as signal transducer and activator of transcription-3 (STAT3), is the name given to the transcription factor that binds to DNA. Target gene promoters contain an interferon-gamma activated sequence (GAS), which is bound by active STAT3 upon translocation into the cell nucleus to control gene transcription [14]. There is often constitutive activation of STAT3 in breast cancer tissues and cell lines. It has been determined that STAT3 is a proto-oncogene since an active form of it can induce tumour growth in nude mice and carcinogenic shift in cultured cells [15]. STAT3 has a broad effect on cellular function, which can be attributed to its numerous identified gene targets. Indirect gene regulation is possible for STAT3 either by regulating the production of other transcription factors or through physically interacting with other transcription factors to either increase or reduce their function [16]. Our research's current objective is to use carboplatin to target the STAT3 gene in the MCF7 breast cancer cell line and evaluate any potential suppression of the cell line's migration and proliferation.

Materials and Methods

Cell Culture and Treatment

The most widely researched human breast cancer cell line in the world, MCF7 was developed from a 69-year-old woman’s pleural effusion who had breast cancer. The NCCS in Pune provided the MCF7 cell line. 1×10^6 human breast cancer cells were seeded into T75 flasks, and the media used was DMEM, which was incubated at 37°C and included 10% FBS, 2 mM glutamine, 0.01 mg/ml insulin, and 1% penicillin/streptomycin mix. Every week, cells need to be passed at a 1:3 sub-cultivation ratio, and two times a week, medium renewal has to be scheduled. DMEM with 10% FBS and the addition of antibiotics and antimycotics was an alternative cell culture medium.

Cell Proliferation - MTT Assay

The MTT test was used to assess the proliferation and vitality of the cells. The MTT test provides an indicator of cytotoxicity, viability, and proliferation in addition to measuring the metabolic activity of cells. The colourimetric test relies on metabolically active cells reducing a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) to purple formazan crystals. Viable cells include enzymes called NAD(P)H-dependent oxidoreductases, which change MTT into formazan. More live, metabolically active cells are found in darker solutions and the colour of the resultant solution obtained by dissolving the insoluble formazan crystals in a solubilization solution and measuring absorbance at 500-600 nm is detected using a multi-well spectrophotometer.

RNA Isolation - TRIzol Method

TRIzol Reagent (Sigma) and the authorised procedure were used to extract the total RNA. The In Invitrogen Life Technologies TRIzol Reagent is a handy tool for separating total RNA from cells and tissues in preparation for PCR analysis. The TRIzol reagent is a monophasic solution of phenol and guanidine isothiocyanate. The TRIzol reagent ruptures cells and dissolves cell components while maintaining RNA integrity during tissue homogenization or lysis. Centrifugation is used to separate the solution's organic and aqueous phases following the addition of chloroform. A technique for recovering RNA that is still in the aqueous phase is isopropanol precipitation. RNA dissolves again in nuclease-free water.

Reverse Transcription

"cDNA synthesis" describes the reverse transcription method used to create cDNA. Reverse transcriptase, mRNA or miRNA, and a thermostable primer complementary to the 3' end of the mRNA or miRNA template act as the template in this procedure. The generated cDNA products are used as a template for the synthesis of the second DNA strands through the use of PCR techniques.

Gene Expression Analysis

The well-known approach for detecting low amounts of miRNA with great sensitivity is qRT-PCR. It is also the most extensively used technique for assessing gene expression. qRT-PCR, a sensitive and accurate method of measuring miRNA expression, can be used to achieve absolute quantification. This test was performed to see whether any genetic or molecular changes were caused by the medication.

Statistical Analysis – SPSS

The data presented the mean of the subsequent experiments +SEM. The statistical difference between the groups was ascertained using the student T-test (programmes in Microsoft Excel 365). Using a one-way ANOVA in SPSS, all the data were analysed, and the mean SD for three duplicates were provided as the result. P 0.05 was chosen as the threshold for statistical significance.

Results

Microscopic Analysis of Breast Cancer Cells

The MCF-7 cell line's breast cancer cells exhibit unique properties when examined under a microscope, most notably the presence of keratin clumps, which are a sign of breast cell carcinoma. Using a thorough microscopic analysis, the process of keratinization is shown as a crucial element involved in the development and proliferation of these cells. By providing an in-depth understanding of the morphological aspects that define the pathological condition in the MCF-7 cell line, this histology research (Fig.1) offers vital insights into the complex microscopic properties associated with breast cancer.

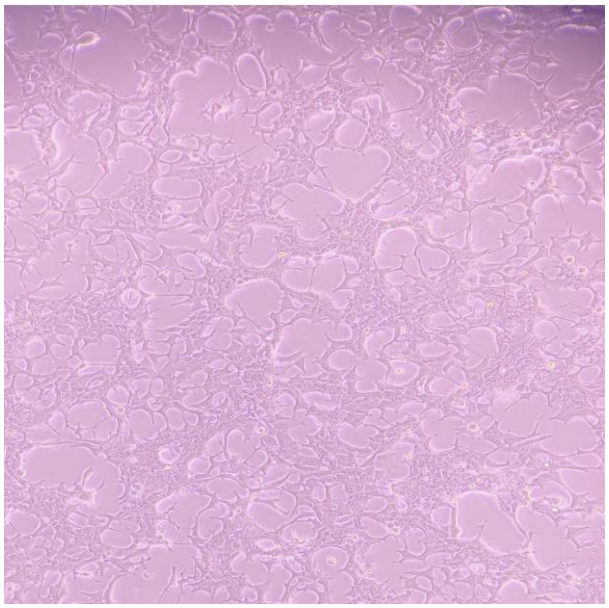

Figure 1 represents the MCF7 Breast cancer cell line. The cell line morphology is similar to that of epithelial cells, and the fluid accumulation between the cell monolayer and culture dish causes monolayers to form dome structures

Dynamics of MCF-7 Breast Cancer Cell Proliferation During Carboplatin Treatment

Analyzing proliferation rates in detail offers important insights into the functional effects of cisplatin on MCF-7 cells. A clear story is told by the line graph (Fig. 2) that compares the dynamics of cell proliferation before and after carboplatin treatment. It clearly shows that MCF-7 cells' ability to proliferate was significantly reduced after treatment. This discovery highlights the strong inhibitory impact of carboplatin on the unchecked proliferation feature of cells, offering a potentially effective therapeutic approach. The observed alteration in the dynamics of proliferation highlights the potential of carboplatin as a promising medication for targeted therapeutic techniques in the treatment of breast cancer by preventing the aberrant cell division associated with the disease.

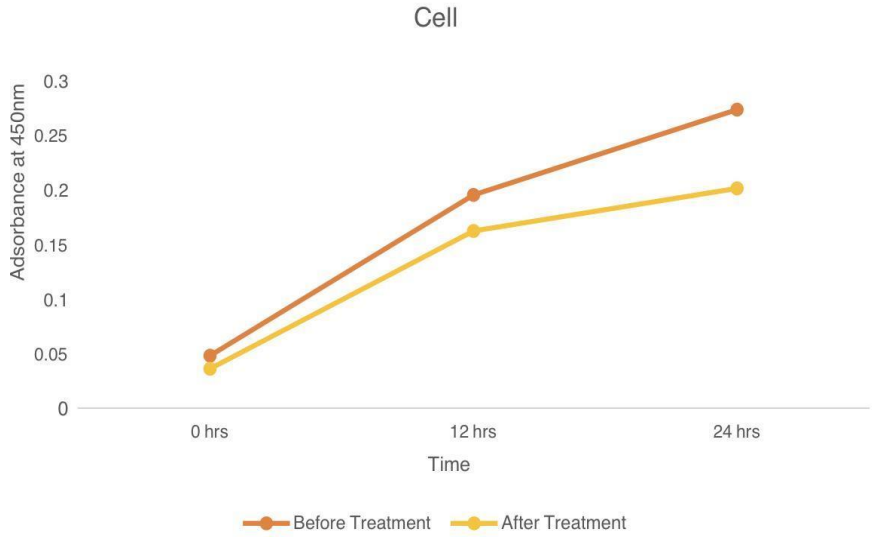

Figure 2 represents the cell proliferation of MCF7 cells before and after treatment with carboplatin. The proliferation rate has significantly decreased after treatment with carboplatin

Carboplatin-Induced Molecular Alteration of STAT3 Gene Expression in MCF-7 Breast Cancer Cells

Going even further into the molecular domain, Fig 3's gene expression analysis concentrates on the STAT3 gene in MCF-7 cells that have received carboplatin treatment. In contrast to typical breast cancer cells, the graph shows a considerable decrease in STAT3 expression after carboplatin therapy. This molecular modulation illuminates the complex effects of carboplatin on important signalling pathways linked to the development of inflammation and cancer in MCF-7 cells. A direct effect on cell proliferation as well as a significant impact on the molecular makeup of breast cancer cells are suggested by the observed drop in STAT3 expression, which is consistent with the carboplatin's wider therapeutic opportunities. With complications for focusing on molecular pathways essential to the advancement of breast cancer, this molecular discovery enhances carboplatin's potential as a versatile medicinal agent.

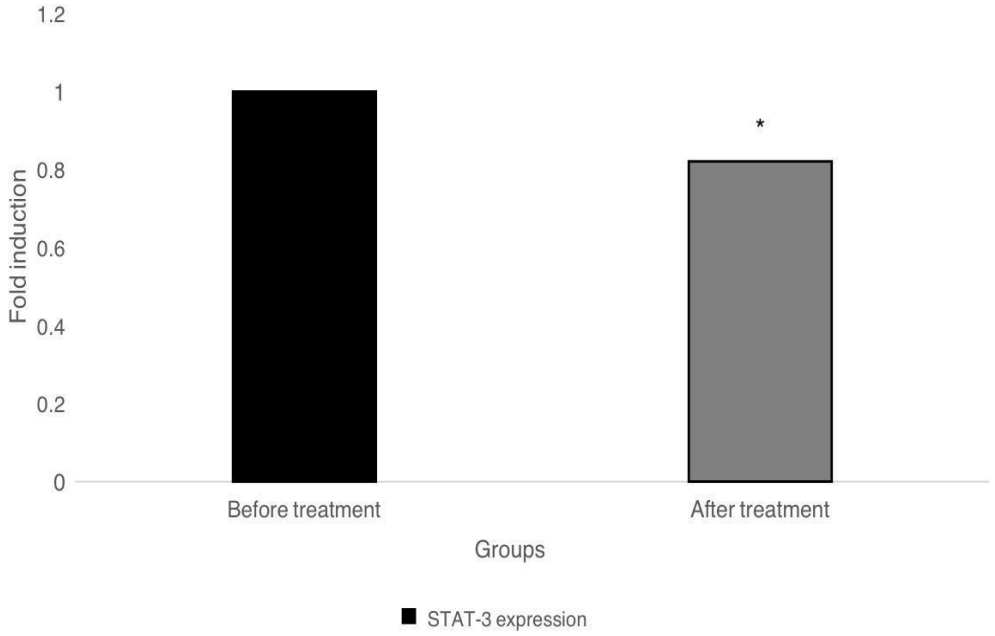

Figure 3 represents the expression levels of STAT3 before and after treatment of MCF7 with Carboplatin. The expression of STAT3 was significantly decreased after the treatment when compared to the normal breast cancer cells.

Discussion

To date, no research has been done on the use of carboplatin targeting the STAT3 gene to prevent breast cancer from proliferating and migrating. On the other hand, a wealth of data supports the effectiveness of carboplatin. According to research by Ke-Da Yu, paclitaxel + carboplatin might be an alternate adjuvant chemotherapy strategy for patients with operable triple-negative breast cancer [17]. According to Edith A. Perez's research, several current studies back up the usual practice of using carboplatin as the first-line treatment for metastatic breast cancer. Other phase II and III trials are looking into various carboplatin combinations for patients selected based on the HER2 status of their malignancy. In metastatic illness, the combination of trastuzumab and carboplatin/taxane therapy has demonstrated good first outcomes [18].

Conclusion

The results indicate that carboplatin, via targeting the STAT3 gene, has a significant inhibitory effect on the migration and proliferation of the MCF7 breast cancer cell line. Additional research is necessary to

determine the ideal IC-50 dosage for carboplatin, conduct a thorough assessment of its effectiveness, and obtain approval for clinical trials, as this work is preliminary. The need for a more thorough investigation to confirm and clarify the possible therapeutic advantages of carboplatin in the context of treating breast cancer is highlighted by this, opening the door for future research and possibly clinical practice integration.

Conflict of Interest

The authors declare there is no conflict of interest.

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