Asian Journal of Medical Research & Health Sciences, vol 2, issue 03, 2024; p50-55,
doi: 10.5455/AJMRHS.1107202300028, E-ISSN 2583-7761

Original Article-Cancer

# ANALYZING STAT-3 GENE EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA CELLS PRE- AND POST-CARBOPLATIN TREATMENT IN VITRO

S Aashruthaa[1], Dhanraj Ganapathy[1], Ameya K P[2], Ashikha Shirin Usman P P[2],Durairaj Sekar[2]*
[1]Department of Prosthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical And Technical Science (SIMATS), Saveetha University, Chennai, India
[2]RNA Biology Lab, Saveetha Dental College and Hospital, Saveetha Institute of Medical And Technical Science (SIMATS), Saveetha University, Chennai, India
***Corresponding Author**: Dr. Durairaj Sekar, Professor (Research), RNA Biology Lab, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77 Email id: duraimku@gmail.com, durairajsekar.sdc@saveetha.com

**Article received on:** 03-06-2024 **Article accepted on:** 17-07-2024 **Article published on** :02-09-2024

## ABSTRACT

**Aim:** Oral squamous cell carcinoma (OSCC) represents a small percentage of global cancer cases, with oral tumors being the most common. OSCC can be caused by various factors including tobacco, alcohol, and viral infections. Carboplatin, a platinum-based chemotherapy drug, shows promise in treating OSCC with fewer side effects. However, activation of Signal Transducer and Activator of Transcription 3 (STAT3) in OSCC cells can hinder carboplatin's effectiveness. This study aims to assess carboplatin's efficacy in OSCC treatment and explore the potential benefits of combining it with STAT3 inhibitors. **Materials and methods:** KB cells were subjected to treatment with varying concentrations of carboplatin. Cell proliferation was evaluated through the MTT assay, while RNA isolation was performed using the TRIzol method followed by cDNA conversion. Gene expression analysis was carried out using qRT-PCR. Statistical analysis of the results was conducted using SPSS. **Results:** The significant reduction in STAT3 expression post-treatment compared to untreated cancer cells indicates a promising avenue for further exploration of carboplatin's therapeutic efficacy in targeting oncogenic signaling pathways. **Conclusion**: The results show a significant decrease in STAT3 expression post-treatment compared to untreated OSCC cells, suggesting carboplatin's strong anti-cancer effect and its potential as a therapeutic option for OSCC treatment.

**Key Words:** Oral squamous cell carcinoma, Carboplatin, STAT3, expression, treatment

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) presents a complex landscape within the context of oncology,Crepresenting 2-4% of cancer cases worldwide, with over 90% of all oral neoplasms falling under OSCC(1). Arising predominantly within the oral cavity, OSCC poses a formidable challenge due to its tendency for slow onset and therefore, it often goes unnoticed in the early stages. Contributing to its clinical complexity are the risk factors implicated in its pathogenesis, ranging from conventional carcinogens such as snuff, alcohol consumption and tobacco smoking to emerging viral culprits such as human papillomavirus (HPV) and hepatitis C virus (HCV)(2).This underscores the intricate interplay of genetic, environmental and lifestyle determinants in driving OSCC development and progression. The journey from benign oral lesions to invasive OSCC include a cascade of molecular events marked by dysregulated cell cycle dynamics, aberrant signaling pathways, and unchecked cellular proliferation. Precancerous lesions serve as indications of malignant transformation, signalling towards invasive disease characterized by local tissue invasion and distant metastasis. At the heart of OSCC pathogenesis lies the disruption of cellular homeostasis, composed by a complex interplay of genetic mutations, epigenetic alterations, and microenvironmental that leads uncontrolled tumor growth and dissemination(3,4).

Carboplatin is a chemotherapy medication frequently employed in treating a rage of cancers,

including OSCC. OSCC stands as a prevalent form of oral cavity malignancy, frequently linked to risk factors like tobacco and alcohol consumption, as well as HPV infection(5). The therapeutic effect of carboplatin in OSCC relies on its capability to inhibit the growth and spread of cancer cells. It falls within the category of drugs referred to as platinum-containing compounds, and it works by interfering with the DNA in cancer cells. Carboplatin exerts its therapeutic effect by forming covalent bonds with DNA molecules within the cancer cells. This binding disrupts the DNA structure, preventing the cells from properly replicating and repairing their DNA. As a result, the cancer cells become unable to divide and proliferate, ultimately leading to their death(6). Carboplatin is commonly used as part of combination chemotherapy regimens for the treatment of OSCC, either as a primary treatment or as part of adjuvant therapy following surgery or radiation therapy. It may be administered intravenously in a clinical setting(7). The therapeutic efficacy of carboplatin in OSCC is well-documented in clinical studies. It can be effective in reducing the size of tumours, controlling the spread of cancer, and improving overall survival rates in patients with advanced or metastatic OSCC(8).

Signal Transducer and Activator of Transcription 3 (STAT3) is a transcription factor that plays a crucial role in regulating gene expression involved in cell proliferation, survival and immune evasion (9). In OSCC, STAT3 is often constitutively activated, promoting tumor growth, invasion and metastasis. Studies suggest that STAT3 activation can confer resistance to chemotherapy agents like carboplatin in OSCC cells. By promoting anti-apoptotic pathways and enhancing DNA repair mechanisms, activated STAT3 can decrease the effectiveness of carboplatin treatment, leading to reduced treatment response and poorer patient outcomes. Understanding the interplay between STAT3 signaling, carboplatin resistance, and OSCC progression is essential for developing more effective therapeutic strategies (10). Targeting STAT3 signaling pathways in combination with carboplatin treatment may represent a promising approach to overcome resistance mechanisms and improve treatment outcomes for OSCC patients.

## MATERIALS AND METHODS

### Tissue culture and carboplatin treatment

KB cell lines were obtained from the Department of Oral Surgery, Saveetha Dental College and Hospital and cultured in the ideal conditions required for its growth. The cells were treated with carboplatin(cis-diammine(cyclobutane-1,1-dicarboxylate-O,O')platinum(II)) for 48 hours.

### Cell proliferation- MTT assay

Following a 24-hour treatment with varying doses of carboplatin, the medium containing an MTT reagent (IL-6 gene) was added back to the cultured KB cells. The purple-blue formazan crystals were placed in an incubator set at 37℃ for a duration of four hours. After dissolving them in dimethyl sulfoxide (DMSO), the absorbance at 570 nm was determined(11). Changes in morphology were seen under a microscope. Based on the MTT assay results, the inhibitory concentrations were utilized to determine the IC 50 dose of carboplatin for the ensuing experiments.

### RNAisolation

Malignant and normal tissue samples were treated for RNA extraction utilising the TRIzol reagent (Invitrogen, Carlsbad, USA) as per the manufacturer's guidelines. The purity and concentration of the RNA were measured using Nanodrop 2000 Lite spectrophotometry (Thermo Fisher Scientific, Waltham, MA). The RNA was then stored at -20℃ for further analysis(12).

### Reverse transcription

Real-time PCR settings and reverse transcription have been previously documented. The following were STAT 3 primers:

5′-CATATGCGGCCAGCAAAGAA-3′ (forward)

5′-ATACCTGCTCTGAAGAAACT-3′ (reverse)

### Statistical analysis

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The facts were shown as average standard deviation (SD). The student used a t-test program to compare the gene expression in cancerous and normal close by tissue. $P \leq 0.05$ was decided to be important in the field of statistics.

## RESULTS

### Histological description of OSCC (KB) cells during in vitro cultivation

Microscopic analysis of OSCC cells (KB) provides a comprehensive understanding of their unique characteristics, such as squamous differentiation, as depicted in **Figure 1**. This differentiation is marked by the notable presence of keratin and the development of intercellular bridges, reflecting the cellular morphology typical of OSCC. When cultivated in vitro, these cells demonstrate strong adherence to the culture flask, forming a cohesive monolayer that accurately mimics their natural growth patterns observed in vivo.

### Proliferation Dynamics of KB Cells under Carboplatin Treatment

In examining the functional impact of carboplatin on KB cells, a thorough evaluation of proliferation rates yields valuable insights. The line chart (**Figure 2**) illustrating cell proliferation dynamics before and after carboplatin treatment reveals a compelling narrative, clearly indicating a significant decrease in the proliferative capacity of KB cells following treatment. This observation underscores carboplatin's potent inhibitory effect on the unchecked growth typical of cells, offering a promising avenue for therapeutic intervention. The observed changes in proliferation dynamics highlight carboplatin's potential to disrupt the abnormal cell division associated with OSCC, emphasizing its role as a promising agent for targeted therapeutic approaches in OSCC treatment.

### Molecular Modulation of STAT-3 Gene Expression in KB Cells by Carboplatin

Further exploring the molecular dimension, the gene expression analysis showcased in **Figure 3** focuses on the STAT-3 gene within carboplatin-treated KB cells. The graph visually depicts a notable decrease in STAT-3 expression post-carboplatin treatment compared to untreated OSCC cells. This molecular adjustment illuminates carboplatin's intricate impact on critical signaling pathways associated with inflammation and cancer advancement within KB cells. The observed reduction in STAT-3 expression aligns with the broader therapeutic implications of carboplatin, suggesting not only a direct effect on cell proliferation but also a significant influence on the molecular landscape of OSCC cells. This molecular insight bolsters the potential of carboplatin as a versatile therapeutic agent, with implications for targeting molecular pathways pivotal to OSCC progression.

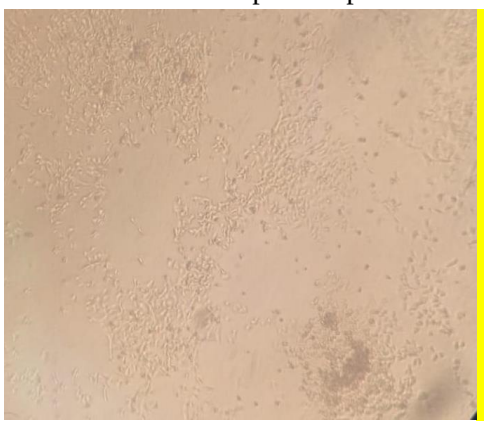

**Figure 1**: Microscopic image shows the cells of oral squamous cell carcinoma (KB).

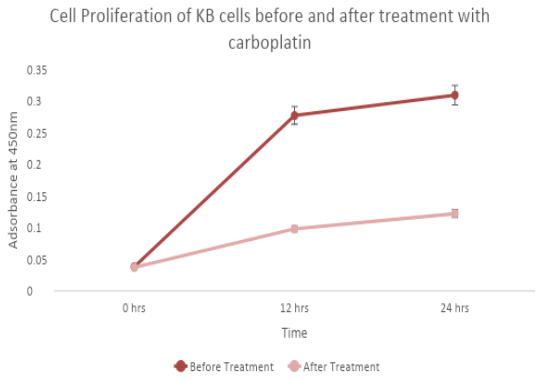


**Figure 2:** Proliferation rates of cells before and a treatment with carboplatin

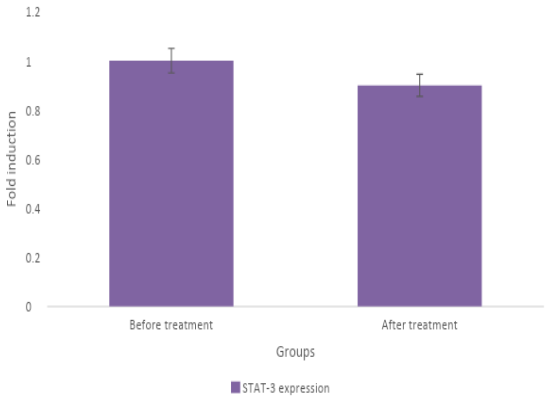


**Figure 3**: Expression of STAT-3 gene before and after treatment of KB cells with carboplatin

**Figure 1**: Microscopic image shows the cells of oral squamous cell carcinoma (KB).In this microscopic observation, OSCC demonstrates squamous differentiation through the formation of keratin and intercellular bridges. When cultured in vitro, these cells adhere to the culture flask and form a monolayer.

**Figure 2** illustrates the proliferation rates of cells before and after treatment with carboplatin and the graph shows the proliferation rate of KB cells has significantly reduced after treatment with carboplatin

**Figure 3** shows the expression of STAT-3 gene before and after treatment of KB cells with carboplatin is seen. This graph represents that the expression of STAT-3 was significantly reduced after the treatment with carboplatin when compared to the normal OSCC cells.

## DISCUSSION

OSCC refers to a cancer type that develops in the squamous cells lining the mouth and throat. Squamous cells are flat, thin cells that form the lining of the mouth, throat, and other structures. OSCC is the predominant form of oral cancer, representing over 90% of all oral malignancies. The primary risk factors for OSCC include tobacco use and excessive alcohol consumption. The risk is significantly higher in individuals who use both tobacco and alcohol.Recently, there has been an increasing association between certain strains of HPV (especially HPV-16) and the development of oral cancers, including OSCC.Research is still being done in the areas of determining predictive biomarkers for treatment response, enhancing patient selection standards, and investigating innovative combination therapies(13).Carboplatin is a chemotherapy medication containing platinum, frequently employed in treating diverse cancers such as ovarian, lung, head and neck, and testicular cancers. It belongs to the platinum-based class of cytotoxic drugs, which also includes cisplatin and oxaliplatin (14). It is considered a second-generation platinum agent and was developed to address some of the toxicity issues associated with cisplatin. Carboplatin exerts its therapeutic effects by forming DNA adducts, leading to DNA cross-linking and followed by suppression of DNA replication and transcription. In OSCC, which is characterised by uncontrolled cell proliferation, this mechanism can help disrupt the rapid growth of cancer cells(6), (15).

Numerous clinical studies have investigated the use of carboplatin in OSCC treatment, either alone or in conjunction with other chemotherapeutic medications (16). These studies have reported varying response rates and survival outcomes. Some patients exhibit a positive response to carboplatin, experiencing tumour shrinkage and prolonged progression-free survival. However, not all patients respond equally, and factors such as tumour stage, molecular subtype, and overall health

can influence treatment outcomes.While carboplatin can be effective in treating OSCC, it is not without side effects. Common adverse events include myelosuppression (decreased blood cell production), nausea, vomiting, and nephrotoxicity. The management of these side effects is crucial for maintaining the patient's quality of life during treatment. Dose adjustments and supportive care strategies are often employed to minimise the impact of adverse events(17).

STAT3 is a transcription factor, which means it regulates the expression of genes by binding to DNA.Dysregulation of STAT3 signalling in immune cells can contribute to conditions such as autoimmune diseases and chronic inflammation(9,10).Understanding the intricate signaling pathways involving proteins like STAT3 is crucial for developing targeted therapies in cancer and other diseases where dysregulation of these pathways occurs.The results show significant reduction in STAT3 levels,suggesting that there is inhibition of cancer cell growth.

While carboplatin has proven efficacy in various cancers, its use is not without limitations. Resistance can develop, and its effectiveness may vary among different tumor types and individual patients. Ongoing research aims to understand the mechanisms of resistance and identify ways to enhance the drug's therapeutic impact.

## CONCLUSION

The findings suggest the expression of STAT3 was notably decreased following treatment in comparison to normal OSCC cells. Therefore, carboplatin has significant anti-cancer effect and therapeutic potential on OSCC cells.Further studies in vivo could explore more therapeutic effects of carboplatin. Later, clinical trials could be done for its efficacy on human beings as a chemotherapy drug for OSCC.

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