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In Vitro analysis of TNF-alpha with the drug Paclitaxel in OSCC cells before and after the treatment

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ABSTRACT
OBJECTIVE To Invitro analysis of the drug Paclitaxel in Oral Squamous Cell Carcinoma cells.Oropharyngeal cancer, also known as Oral Squamous Cell Carcinoma (OSCC), is a cancer derived from squamous cell in the oral cavity or oropharyngeal. Globally, it is a major public health problem whose incidence has gone up greatly. For the treatment of OSCC, a multi discipline approach is used such as surgery, radiotherapy and chemotherapy. The use of Paclitaxel in fighting OSCC has been recently investigated by researchers. Therefore, in vitro studies help in testing the potential effects of Paclitaxel on the OSCC (oral squamous cell carcinoma) cells, in a strictly controlled environment. Such studies shed light on how the drug works, its anti-tumor activity, and its utility as an adjuvant in OSCC therapy.
MATERIALS AND METHODS Cell culture and treatment involves the following steps:KB cells were treated with paclitaxel.Cell proliferation was performed by MTT assay RNA isolation is made using Trizol method .cDNA conversion has been conducted. Real time- polymerase chain reaction was used for the analysis of gene expression Statistical analyses were done using SPSS software.Culture of the tissues and cisplatin treatment:OSCC cells were obtained from the Department of Oral Surgery at Saveetha Dental College and Hospital, and they were grown following standard guidelines .In the drug treatment experiments, cells underwent paclitaxel for 48 hours.
RESULT In this microscopic view OSCC exhibits squamous differentiation by formation of keratin and intercellular bridges. When grown in vitro, these cells stick to the culture flask and develop into a monolayer.. The proliferation rate has significantly reduced after treatment with packitaxel.The expression of TNF-a was significantly reduced after the treatment when compared to the normalKB cells
CONCLUSION Paclitaxel reduces the proliferation of kB cells.It drastically reduce cell proliferation within the first 12 hrs of treatment and later plateau state was observed.Thus we conclude that TNF- alpha is reduced in the presence of paclitaxel in OSCC cells

KEYWORD Paclitaxel,Oral Squamous Cell Carcinoma (OSCC),Cell Culture,Drug Treatment,Cell Viability,Apoptosis Assays,Cytotoxicity,MTT Assay

INTRODUCTION

Oropharyngeal cancer, also known as Oral Squamous Cell Carcinoma (OSCC), is a cancer that arises from squamous cells in the oral cavity or oropharynx. Such studies shed light on how the drug works, its anti-tumor activity, and its utility as an adjuvant in OSCC therapy.(Han *et al.*, 2016)Globally, it is a major public health problem whose incidence has gone up greatly. (Blumenthal, 2008; 'Reviewing the potential application of miR-21 inhibitors in oral cancer therapeutics', 2022).For the treatment of OSCC, a multidisciplinary approach is used, including surgery, radiotherapy, and chemotherapy..(Parsons, 2007)Researchers have studied the effectiveness of Paclitaxel in combating OSCC.
Tumor necrosis factor-alpha (TNF alpha), from the macrophages/monocytes of the body when inflammation occurs in the tissue, can regulate multiple types of signaling events inside cells and leads to necrosis or apoptosis. The protein also plays an important role by enhancing immunity to disease, both from bacteria and cancer. TNF alpha is known to be linked to a wide range of diseases, including cancer, according to scientists. the effectiveness of TNF in cancer rests on its local application concentrated on the aggressive local soft tissue sarcomas, metastatic melanomas, and unresectable tumors of any histology to save the limb from amputation.
Therefore, in vitro studies help in testing the potential effects of Paclitaxel on the OSCC (oral squamous cell carcinoma) cells, in a strictly controlled environment(Wasan and Badea, 2019) Such studies shed light on how the drug works, its anti-tumor activity, and its utility as an adjuvant in OSCC therapy.(Almeida *et al.*, 2023)Paclitaxel derived from the bark of the pacific yew tree have been extensively used for therapeutic purposes in managing solid tumors in cancer patients e.g., breast, ovarian and lung cancers.(Blumenthal, 2008) It acts via stabilizing of a microtubule that inhibits normal cell mitosis leading its blockage.(*September 2021 - Volume 74 - Issue 3 : Hepatology*, no date) Although Paclitaxel has proven helpful in diverse cancer types, it needs specific evaluation in regard to OSCC management.(Bocci and Francia, 2014)
There is also a reason that Paclitaxel should be explored for treatment of this aggressive type of oral cancer. According to prior research, OSCC tumors are a diverse species that respond differently to standard treatments.(Reinhold and Tilgen, 2002; Blumenthal, 2008) Therefore, a precise study of Paclitaxel's influence on OSCC cells must be conducted to establish

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its clinical efficacy in this scenario.(‘Expression analysis of transforming growth factor beta (TGF-β) in oral squamous cell carcinoma’, 2024)Several cancers have been extensively studied on how paclitaxel stabilizes microtubules and promotes cell cycle arrest.(Yuzhalin and Kutikhin, 2014) Thus, its particular implications for OSCC cells should be studied independently due to their distinct genetic and molecular traits as oral cancers. In this manner, we hope that our findings can enlighten researchers in future studies relating to OSCC cell responses towards paclitaxel as well as the potential therapies involving this chemotherapeutic agent.(‘Circulatory microRNAs inhibition and its signaling pathways in the treatment of oral squamous cell carcinoma (OSCC)’, 2022)

MATERIALS AND METHODS

Culture of the tissues and pacitaxel treatment

OSCC cells were obtained from the Department of Oral Surgery at Saveetha Dental College and Hospital, and they were grown following standard guidelines .In the drug treatment experiments, cells underwent paclitaxel for 48 hours.

Cell culture and treatment involves the following steps:

KB cells were treated with paclitaxel.Cell proliferation was performed by MTT assay RNA isolation is made using Trizol method .cDNA conversion has been conducted. Real time- polymerase chain reaction was used for the analysis of gene expression Statistical analyses were done using SPSS software.

CELL PROLIFERATION- MTT assay

24-hour treatment of cultured OSCC cells with various concentrations of boldine and the medium, including an MTT reagent KBcells back. For four hours, the purple-blue crystals of formazan were incubated at 37 °C. DMSO was then used to dissolve and the absorbance at 570 nm recorded . Microscopic changes were observed. Based on the outcomes of MTT assay, it was possible to determine what dose of boldine should be used for the following experiments utilizing inhibitory dosages.

RNA ISOLATION

Total RNA was isolated from OSCC cells using the Trizol reagent (Invitrogen) according to the manufacturer’s instructions. The purity and concentration of the extracted RNA were determined using a Nanodrop 200 Lite spectrophotometer (Thermo Fisher Scientific, Waltham , MA).

REVERSE TRANSCRIPTION:

Real-time PCR settings and reverse transcription have been previously documented The following were primers: AGTGAGGAACAAGCCAGAGC in forward and GTCAGGGG-TGGTTATTGCAT in reverse

Gene expression analysis – qRT-PCR:

The total RNA was extracted using the TRIZOL reagent. For one-stranded cDNA synthesis, two units of total RNA dT primer and SII RT were used. qRT-PCR was performed on an iCycler using validated primers, and SYBR Premix Ex Taq II. We quantitated the transcripts of specific genes of interest by setting a cycle-number threshold. The transcripts were normalized during the same incubations using GAPDH as a reference gene.

STATISTICAL ANALYSIS

The statistical analysis was done using SPSS software. The data was presented using the mean ± SD format. The gene expression level in adjacent normal tissues and cancerous tissues was compared using an automated t-test program. P = 0.05 was discovered to be statistically significant.

RESULT

Histomorphological Characterization of OSCC Cells (KB) in In Vitro Cultivation

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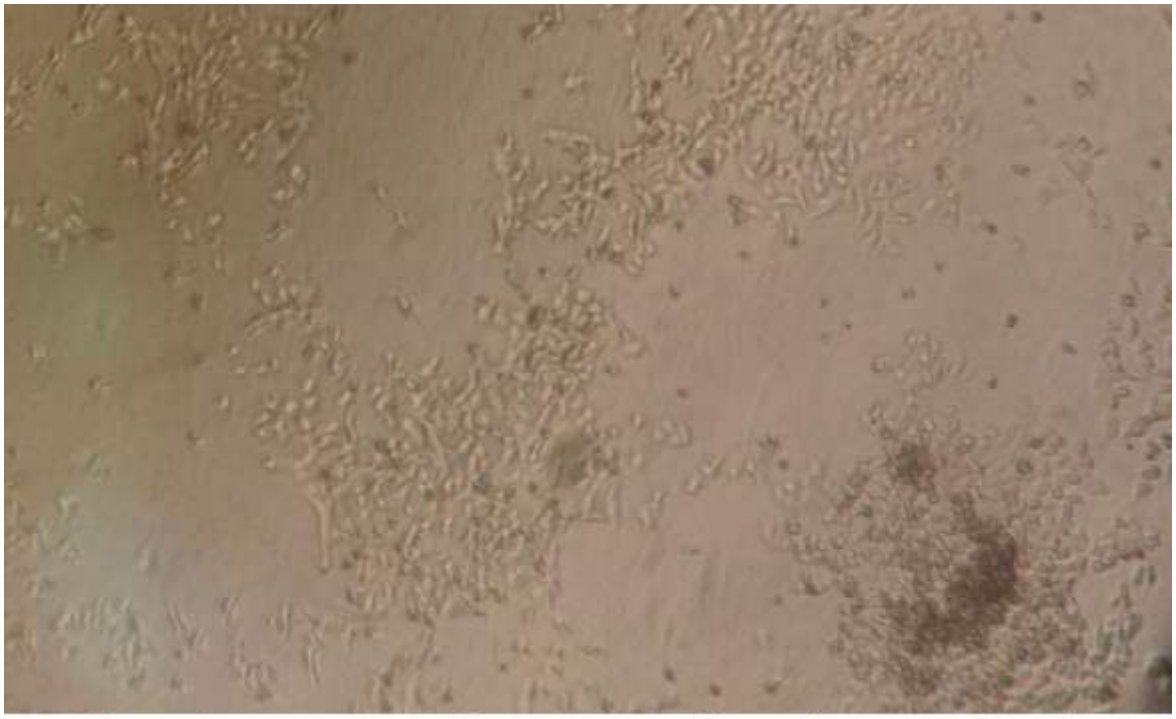

Figure 1 represents oral squamous cell carcinoma (KB) cells

Figure1:Histomorphological Characterization of OSCC Cells (KB) in In Vitro Cultivation

In this microscopic view OSCC exhibits squamous differentiation by formation of keratin and intercellular bridges as shown by (Fig.1) . When grown in vitro, these cells stick to the culture flask and develop into a monolayer.Cellular and Tissue Characterization of Squamous Cell SCC Lines (KB) of OSCC in Laboratory Cultivation.The tiny structures of the cells cultured in vitro (KB) allows us to see with great clarity the specific attributes of cellular maturation. The fact that this monolayer (KB) is tightly expressing itself to the culture flask demonstrates their ability to adapt properly to the natural growth patterns found in vivo.

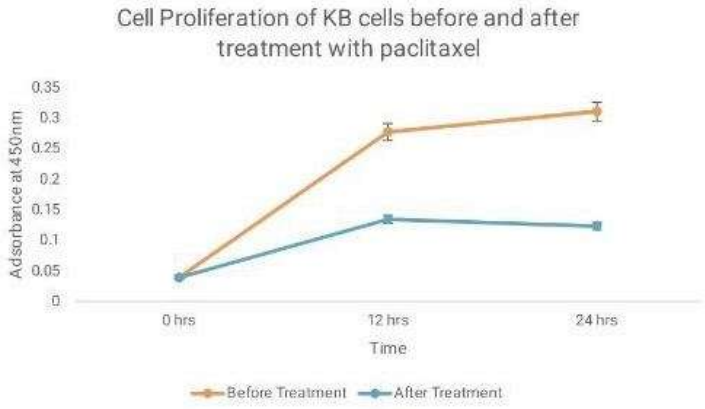

Figure 3: paclitaxel -Induced Effect on KB cell proliferation and Molecular Signals in OSCC

The line chart represents the cell proliferation of KB cells before and after treatment with cisplatin. The proliferation rate has significantly reduced after treatment with packitaxel.While the treatment was ongoing, the bar graph (Fig.2) that illustrates the rate of KB cell proliferation shows that this rate decreases significantly. The highlighted observation therefore testifies the vital importance of paclitaxel in blocking what is defined as the uncontrolled growth which is a notorious feature of the oral squamous cell carcinoma. The cytostatic effect on cell proliferation, shown by the graph, have therapeutic consequences giving the prospect for paclitaxel as an effective antineoplastic agent in this kind of cancer.

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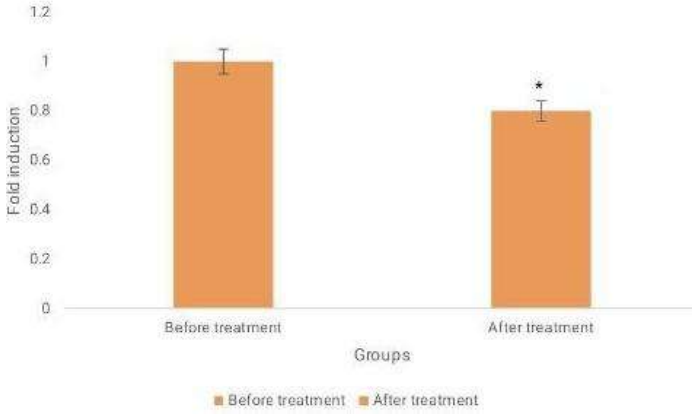

Figure3:Expression analysis TNF-a before and after treatment with paclitaxel

Figure 3 represents the expression levels of TNF-a before and after treatment of KB cells with paclitaxel. The expression of TNF-a was significantly reduced after the treatment when compared to the normalKB cells converting the rhetoric scope to molecular form, the following bar graph (fig.3) reveals the expression of TNF-a before and after paclitaxel administration. The X axis represents both treatment time before and after therapy, and the Y axis represents fold change over control. Relevantly, the sharp drop in the standard levels of TNF-a after the treatment procedure indicates the modulation of crucial inflammation and cancer-advancing signaling pathways. Thus, the observed result backs up the assumption that cisplatin shows not only direct effects on cell proliferation, but cell growth is modulated through mediation of TNF-a expression.

DISCUSSION

Determine the cytotoxicity of Paclitaxel in OSCC cells: Assess the cytotoxicity of Paclitaxel in OSCC cells. The primary objective is to determine how effective Paclitaxel is in killing OSCC cells.(Panta, 2019; Almeida *et al.*, 2023)This would involve assessing the drug's impact on cell viability, proliferation, and induction of cell death (apoptosis) in OSCC cell lines. Assess the safety and side effects of paclitaxel in OSCC cells. Paclitaxel can have side effects and toxicity profiles that may vary in different cancer cell types.(Sawatani *et al.*, 2020; Kii, Sakuma and Tanaka, 2021a)Combination therapies: The effectiveness of Paclitaxel can be enhanced and treatment outcomes for OSCC patients can be improved by combining chemotherapy drugs, targeted therapies, immunotherapies, or radiation therapy. (Kii, Sakuma and Tanaka, 2021a)Mechanisms of resistance: Investigate the molecular mechanisms underlying resistance to Paclitaxel in OSCC cells. (Mallery *et al.*, 2019)Identifying specific pathways that contribute to resistance can lead to the development of strategies to overcome the resistance.(Kumar *et al.*, 2021)(Song *et al.*, 2019)

Our observations align with those of other researchers who demonstrate the effectiveness of Paclitaxel in various tumors.(Blumenthal, 2008)Nevertheless, this needs to be considered in the context of a high degree of cellular diversity present among cancer cells, requiring additional studies dedicated solely to oral squamous cell carcinoma (OSCC).The importance of considering specific cell types is highlighted by the research that has shown that paclitaxel exhibits different responses in various cancer cell lines.(Blumenthal, 2008; *Carcinogen-Driven Mouse Models of Oncogenesis*, 2021).The recorded changes over period depict the changing character of cell's reaction to this medicament.(Mallery *et al.*, 2019) Since this temporal factor is very important in designing appropriate treatment regimes, more research should focus on identifying the optimum dosing schedule in order to attain maximum therapeutic benefits as well as minimize side effects of the treatment(Meng *et al.*, 2021)

The study offers insight into creating a personalized treatment plan for head and neck cancers. (Reinhold and Tilgen, 2002)The addition of paclitaxel into combinations including other agents or modalities such as targeted therapy, or immunotherapy might optimize efficacy in this group and should be explored further.(Fribley, 2016)Further studies should seek ways of complementarity Paclitaxel and existing therapeutic approaches in OSCC.(Kii, Sakuma and Tanaka, 2021b))

CONCLUSION

We have finally concluded that our conducted in vitro analysis of paclitaxel in oral tumors has been very useful in identifying new strategies for treatment using this much known anti-cancer drug. The comprehensive evaluation of Paclitaxel's actions in OSCC cells has revealed significant insights that are crucial for determining the potential effectiveness of Paclitaxel, further investigations, and possible treatments. reduces the proliferation of kB cells.It drastically Within the first 12 hours of treatment, there was a reduction in cell proliferation and then a plateau state was observed.(Song *et al.*, 2019; Choi *et al.*, 2020)Thus we conclude that TNF- alpha is reduced in the presence of paclitaxel in OSCC cells(Song *et al.*, 2019)Our study results show that the viability of OSCC cells decreases based on the dose level when exposed to Paclitaxel. This is indicative of the effect that Paclitaxel could possibly have on impeding unrestricted

multiplication which is one of the salient features of the OSCC. The drug's mechanism of action is well-established and causes blockage of the cell cycle by hindering microtubule dynamics.

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