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Evaluating the therapeutic efficacy of curcumin in the management of oral squamous cell carcinoma cell line

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Abstract

Background: This study was conducted for evaluating the therapeutic efficacy of curcumin in the management of oral squamous cell carcinoma.

Material and methods: SCC-25 cells were maintained in Dulbecco's modified Eagle's medium, enriched with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 µg/ml streptomycin, at a temperature of 37°C in a humidified environment consisting of 95% air and 5% CO2. After treatment with curcumin at concentrations of 0, 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L for 24 hours, concentration of 0, 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L for 48 hours. The cells were fixed on ice for 30 minutes using 100% cold ethanol, subsequently washed, and resuspended in a staining buffer (BD, USA) containing 0.05 mg/ml propidium iodide and RNase A for 30 minutes at room temperature in the absence of light. SCC-25 cells were treated with or without curcumin, and Western blot analyses were conducted. All the results were recorded in Microsoft excel sheet and were subjected to statistical analysis using SPSS software.

Results: The inhibition rate at 24 hours at concentration of 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L was 21 percent, 38 percent, 49 percent and 53 percent respectively. The inhibition rate at 48 hours at concentration of 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L was 43 percent, 59 percent, 66 percent and 71 percent respectively. Curcumin demonstrates an inhibitory effect on the invasion of SCC-25 cells. The cells were treated with curcumin for a duration of 24 hours at varying concentrations of 0, 10, and 40 µmol/L. Mean cell number at concentration of 0, 10, and 40 µmol/L was 63.2, 55.7 and 41.9 respectively.

Conclusion: The findings from our research indicated that curcumin effectively inhibited cell number and invasion of SCC-25 cells. These results imply that curcumin influences the proliferation and invasive behaviour of SCC-25 cells.

Keywords: Curcumin, OSCC, Treatment, SCC-25

Introduction

Oral squamous cell carcinoma (OSCC) represents the most prevalent malignant neoplasm within the oral cavity and ranks as the sixth most common cancer globally. Regrettably, the prognosis for patients diagnosed with OSCC remains unfavourable, with approximately 50%

of individuals succumbing within five years, primarily due to recurrent or metastatic disease. Elevated expression of epidermal growth factor receptor (EGFR) has been observed in OSCC cases and is significantly correlated with higher histological grades.[1-4]

The augmented expression and activity of EGFR are linked to tumor proliferation, invasion, and metastasis. Tumors exhibiting positive phosphorylated EGFR (pEGFR) status are associated with a dismal prognosis and a limited response to chemotherapy. Consequently, EGFR has emerged as a critical target in the ongoing research aimed at developing effective cancer therapies.[5,6]

Curcumin, a polyphenolic compound derived from the rhizome of the turmeric plant, has garnered significant attention in recent years for its potential role as a chemopreventive and therapeutic agent against cancer. Research conducted both in vitro and in vivo has demonstrated curcumin's efficacy in treating various cancer types, including lung cancer, colon cancer, breast cancer, and prostate carcinoma. The compound has been found to interact with a wide array of molecular targets, including PKB/Akt, NF-κB, and MAPK. Recent investigations have also suggested that curcumin's anti-cancer properties may be linked to its ability to modulate the expression of EGFR and its associated downstream signaling pathways. Specifically, studies have indicated that curcumin can inhibit the proliferation of human colon cancer cells by downregulating EGFR gene expression through the attenuation of Egr-1 trans-activation activity.[7-9] This study was conducted for evaluating the therapeutic efficacy of curcumin in the management of oral squamous cell carcinoma.

Material and methods

The present study was conducted for evaluating the therapeutic efficacy of curcumin in the management of oral squamous cell carcinoma. SCC-25 cell line were obtained derived from patients with OSCC. SCC-25 cells were maintained in Dulbecco's modified Eagle's medium, enriched with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 µg/ml streptomycin, at a temperature of 37°C in a humidified environment consisting of 95% air and 5% CO2. These cells proliferate as a monolayer and are deemed suitable for experimentation upon reaching approximately 80% confluency. The effect of curcumin on the proliferation of SCC-25 cells was assessed using the MTT assay. MTT dye was introduced to each well following a minimum treatment duration of 4 hours. Cells were plated at a density of 1.5 × 10^5 cells per well in 6-well plates. After treatment with curcumin at concentrations of 0, 10, and 40 µmol/L for 24 hours, the cells were fixed on ice for 30 minutes using 100% cold ethanol, subsequently washed, and resuspended in a staining buffer (BD, USA) containing 0.05 mg/ml

propidium iodide and RNase A for 30 minutes at room temperature in the absence of light. The cell samples were then analyzed using a flow cytometer, and the percentages of cells in various phases of the cell cycle were determined using the Multicycle software provided by the manufacturer. Total RNA was extracted from SCC-25 cells subjected to different treatments using the Trizol reagent, following the manufacturer's guidelines. Additionally, SCC-25 cells were treated with or without curcumin, and Western blot analyses were conducted. All the results were recorded in Microsoft excel sheet and were subjected to statistical analysis using SPSS software.

Results

We evaluated the inhibition effects of curcumin on the proliferation of SCC-25 cells. The cells were exposed to curcumin at different concentrations for 24 and 48 hours, and cell viability was measured by MTT assay. The dose-dependent inhibition of curcumin on cells proliferation was observed. The cell viability was evaluated by MTT assay and the inhibitory effect was expressed as the percentage of viable cells. The inhibition rate at 24 hours at concentration of 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L was 21 percent, 38 percent, 49 percent and 53 percent respectively. The inhibition rate at 48 hours at concentration of 10 µmol/L, 20 µmol/L, 40 µmol/L and 80 µmol/L was 43 percent, 59 percent, 66 percent and 71 percent respectively. Curcumin demonstrates an inhibitory effect on the invasion of SCC-25 cells. The cells were treated with curcumin for a duration of 24 hours at varying concentrations of 0, 10, and 40 µmol/L. Mean cell number at concentration of 0, 10, and 40 µmol/L was 63.2, 55.7 and 41.9 respectively.

Graph 1: Curcumin inhibits the proliferation of SCC-25 cells.

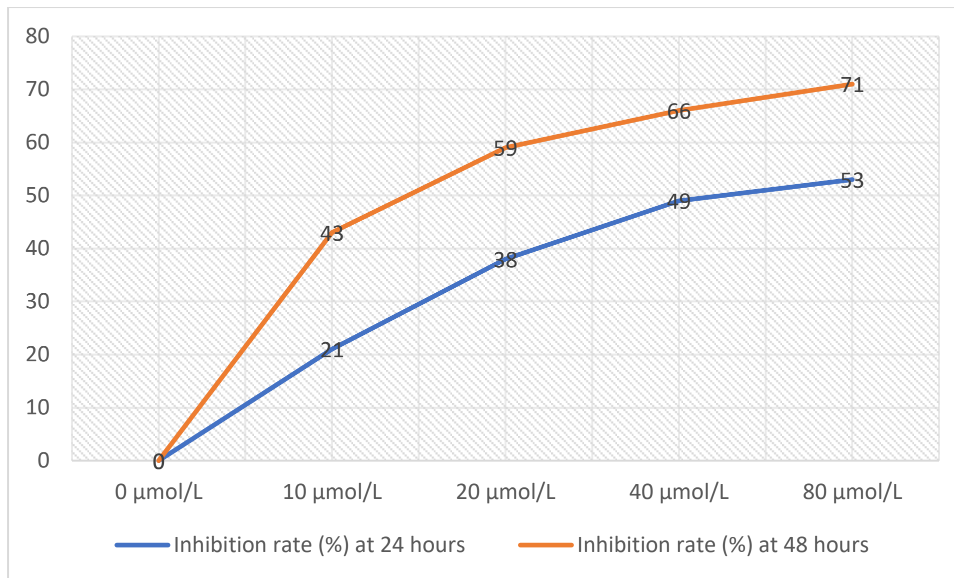

Table 1: Curcumin inhibition of invasion

Concentration (µmol/L)	Mean Cell number
0 µmol/L	63.2
10 µmol/L	55.7
40 µmol/L	41.9

Discussion

Oral squamous cell carcinoma (OSCC) represents one of the most prevalent form of head and neck cancer. Current treatment options for OSCC encompass surgical intervention, which may result in significant functional impairment or cosmetic disfigurement, as well as radiotherapy and chemotherapy. For individuals diagnosed with advanced or terminal stages of the disease, survival durations can be reduced to mere months, underscoring the critical necessity for innovative therapeutic approaches.[10] Curcumin [Cur; 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a naturally occurring phenolic compound widely recognized as the spice turmeric (Curcuma longa), sourced from the rhizome of a plant native to East India. Curcumin has been regarded as pharmacologically safe for consumption in dietary contexts for centuries. Comprehensive studies have demonstrated that Cur possesses a diverse array of biological activities, effectively addressing issues related to aging, inflammation, and cancer. Prior research has indicated that Cur can inhibit the proliferation, metastasis, and initiation of various malignancies, including lung, breast, hepatocellular, pancreatic, and gastric cancers. Furthermore, epidemiological evidence suggests that a diet abundant in Cur may contribute to

a reduced incidence of colon cancer.[11-14] This study was conducted for evaluating the therapeutic efficacy of curcumin in the management of oral squamous cell carcinoma.

We evaluated the inhibition effects of curcumin on the proliferation of SCC-25 cells. The cells were exposed to curcumin at different concentrations for 24 and 48 hours, and cell viability was measured by MTT assay. The dose-dependent inhibition of curcumin on cells proliferation was observed. The cell viability was evaluated by MTT assay and the inhibitory effect was expressed as the percentage of viable cells. The inhibition rate at 24 hours at concentration of 10 μmol/L, 20 μmol/L, 40 μmol/L and 80 μmol/L was 21 percent, 38 percent, 49 percent and 53 percent respectively. The study conducted by Liu T et al aimed to clarify the detailed molecular mechanism though which Cur regulates NF-κB pathway activity in OSCC. The viability of HSC3 and CAL33 cells following treatment with Cur was determined using a Cell Counting Kit-8 assay. The protein and mRNA expression of specificity protein 1 (Sp1), p65 and heat shock factor 1 (HSF1) was determined by western blotting and reverse transcription-quantitative PCR analysis, respectively. The NF-κB activity was measured by Dual-Luciferase reporter assay. Short hairpin RNA targeting Sp1 or control RNA was transfected into HSC3 cells using X-treme GENE HP DNA Transfection System. Colony formation assays were performed using crystal violet staining. The results demonstrated that Cur significantly inhibited the viability and colony formation ability of HSC3 and CAL33 cells. In addition, Cur decreased the expression of Sp1, p65 and HSF1 by suppressing their transcription levels. Cur decreased NF-κB activity in OSCC cells, and Sp1 downregulation enhanced the effect of Cur. The findings suggested that Cur may inhibit the proliferation of OSCC cells via a Sp1/NF-κB-dependent mechanism.[15]

In the present study, The inhibition rate at 48 hours at concentration of 10 μmol/L, 20 μmol/L, 40 μmol/L and 80 μmol/L was 43 percent, 59 percent, 66 percent and 71 percent respectively. Curcumin demonstrates an inhibitory effect on the invasion of SCC-25 cells. The cells were treated with curcumin for a duration of 24 hours at varying concentrations of 0, 10, and 40 μmol/L. Mean cell number at concentration of 0, 10, and 40 μmol/L was 63.2, 55.7 and 41.9 respectively. Zhen L et al investigated the efficacy of curcumin on proliferation and invasion in SCC-25 cell line. They also explored the effect of curcumin on the activition of EGFR and its downstream signaling molecules Akt, ERK1/2 and STAT3. Furthermore, we examined the inhibition effect of curcumin on EGF-induced EGFR phosphorylation and SCC-25 cells invasion. Their results showed that curcumin inhibited SCC-25 cells proliferation and induced G2/M phase arrest in a dose-dependent manner. Curcumin also inhibited SCC-25 cells invasion and downregulated MMP-2, MMP-9, uPA and uPAR expression. Their data showed that

crucumin reduced the EGF-induced phosphorylation of EGFR and suppressed EGF-triggered SCC-25 cells invasion. Taken together, their results suggest that curcumin reduced SCC-25 cells proliferation and invasion through inhibiting the phosphorylation of EGFR and EGFR downstream signaling molecules Akt, ERK1/2 and STAT3.[16]

Conclusion

The findings from our research indicated that curcumin effectively inhibited cell number and invasion of SCC-25 cells. These results imply that curcumin influences the proliferation and invasive behavior of SCC-25 cells.

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