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ANTICARCINOGENIC EFFECT OF MOSINONE-A ON MRNA EXPRESSION OF CELL CYCLE-ASSOCIATED PROTEINS IN DMBA-INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

Employing 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis, we examined how Mosinone-A affected the expression of apoptosis-related proteins. Four groups of ten golden Syrian hamsters each were randomly selected. The untreated controls were animals in Group I. For 14 weeks, Groups II and III's animals received three weekly treatments having 0.5 percent DMBA in liquid paraffin solution on their left cheeks. Starting one week prior to their exposure to the carcinogen, Group III animals received oral doses of Mosinone-A (2 mg/kg body weight). Until the animals were slaughtered, this medication was given on days when no DMBA application had been planned. For the length of the experiment, the animals in group IV were given only Mosinone-A. Cheek pouch carcinomas became apparent following 14 weeks of topical DMBA treatment, together with decreased expression of P12DOC-1, p16INK4A, as well as p15INK4B. Consuming Mosinone-A orally significantly suppressed HBP carcinoma formation as evidenced by overexpression of these genes. The current research's findings indicate that Mosinone-A may have preventive effect against development of DMBA-induced cheek pouch

cancer. This effect appears to be mediated by its ability to inhibit cell proliferation, induce apoptosis, AS WELL AS promote cellular differentiation, thereby exerting its anti-cancer potential.

Keywords: Mosinone-A, Cell cycle proteins, Oral cancer, apoptosis, DMBA, RT-PCR

INTRODUCTION

OSCC (Oral squamous cell carcinoma) has become predominant reason for morbidity as well as mortality globally, having an approximate yearly prevalence of 3,000,000 cases **[1]**. India and several other countries, the high consumption of tobacco and betel quid has been strongly linked to the development of oral cancer. Notably, 95% of oral cancer cases are classified as squamous cell carcinomas **[2, 3]**. The progression of oral cancer entails heightened cellular proliferation, Cellular differentiation dysregulation, prolonged cell survival, along diminished programmed cell death (apoptosis) **[4]**. Current research have highlighted need to control OSCC incidence[5] and have focused on developing effective chemoprevention strategies **[5]**.

The buccal pouch of golden Syrian hamster serves as exemplary animal model for investigating oral carcinogenesis, it consistently develops squamous cell carcinoma upon induction with carcinogen of 7,12-dimethylbenz[a]anthracene **[6]**. It is frequently used to model cheek pouch carcinogenesis in hamsters because DMBA causes highly strong, organ- and site-specific carcinogenesis of the buccal pouch. Dihydrodiol epoxide, DMBA's final carcinogenic metabolite, accelerates carcinogenic procedure by causing chronic inflammation as well as excess ROS (reactive oxygen species) **[7]**. Change status of the previously mentioned markers associated with a number of malignancies, including oral cavity cancer: p12doc-1, p15INK4B, and p16INK4A. P21, nuclear protein referred to as CDK1(cyclin-dependent kinase inhibitor), was extensively present in epithelial cells as well as plays function in cellular maintenance, p53-mediated growth arrest, as well as apoptosis. Consequently, p21 seems to be intricately associated with cancer development **[8]**.

Using RT-PCR analysis, we examined the expression of biomarkers such as P12DOC-1, p16INK4A and p15INK4B, which serve as reliable indicators of cellular proliferation as well as cell cycle control, to evaluate anticarcinogenic effect of Mosinone-A in hamster cheek pouch carcinogenesis. Based on suppression of HBP carcinoma and manipulation of cell proliferation, cell cycle regulation and apoptosis, our results

suggested that cancer cell was strongly inhibited by Mosinone-A.

Mosinone-A. is said to have a much stronger effect than rotenone and. inhibits oxidative phosphorylation of mitochondrial complex I **[9]**. Mosinone-A is a new acetogenin. It has a mono-tetrahydrofuran ring and is cytotoxic to human pancreatic cancer cell lines **[10]**. However, the exact mechanism by which Mosinone-A cytotoxically destroys oral cancer cells is unknown. Therefore, study aimed to examine apoptotic as well as cell cycle regulatory proteins implicated in DMBA-induced carcinogenesis of oral cavity in hamsters.

MATERIAL AND METHOD

Animal

Rajah Muthiah Medical College and Hospital, Annamalai University, has male golden Syrian hamsters at its Central Animal House. The animals were 8to10 weeks old and weighed 80to120g. In Hyderabad, India, they had been obtained from National Institute of Nutrition. The animals received a regular pellet meal, were kept in polypropylene cages, and had unlimited access to water. With a 12h light/dark cycle, temperature, controlled humidity, they had been kept in climate-controlled setting.

Chemicals

In Bangalore, India, Sigma-Aldrich Chemical Pvt. Ltd. supplied the carcinogen DMBA (7,12- Dimethylbenz[a]anthracene). The remaining chemicals had been of analytical grade along side supplied by Bangalore's Himedia Laboratories and Mumbai, India's Sisco Research Laboratories Pvt. Ltd.

Isolation of Mosinone-A

Mosinone-A has been extracted utilizing Maclaughlin method from Annona squamosa bark. [11] Employing ethanol, dried as well as ground bark of Annona squamosa has been extracted. To produce residues which are soluble in hexane, residues began by dividing into fractions that contained 90 percent methanol & hexane, then fractions that contained water and chloroform. Column chromatography was performed on silica gel on the hexane-soluble residue, beginning with a solvent system of hexane as well as chloroform and then a system of methanol & chloroform. To mix resulting fractions, HPTLC analysis was used. Through the use of column chromatography, the mixed fractions were converted into final product, Mosinone-A, a white solid wax. To describe isolated Mosinone-A, LC-MS (Liquid Chromatography-Mass Spectrometry) as well as NMR (Nuclear Magnetic Resonance) had been utilized. Compare it to reference Mosinone-A.

Experimental protocol

The plan for the laboratory was accepted by the school's institutional animal ethics council in Annamalai Nagar, India. Forty golden Syrian hamsters have been separated into four sets of ten for each group at random. The animals in Group I acted as an untreated control. For 14 weeks, Group II and III animals had been given DMBA at 0.5 percent in liquid paraffin to apply to their left buccal pouches three times a week using a No. 4 brush. The animals in Group II didn't get any extra attention. For Group III animals, Mosinone-A (2mg kg-1 b.wt) was given orally 1week prior to animals' carcinogen contact. This was done every other day until the animals were killed. Animals in Group IV were only given Mosinone-A during the experiment. After the fourteenth week of the experiment, all of the animals were put to death by cervical dislocation. Each group's control as well as experimental animals' buccal mucosas underwent Western blotting for protein along with quantitative real-time PCR for mRNA.

Quantitative real-time–PCR

The tissue's total RNA had been isolated employing Qiagen RNase Min Kit. DNase (Qiagen, Valencia, CA) was added to the RNA to eliminate any remaining genomic DNA. To create template cDNAs, Applied Biosystems' Taqman reverse transcription reaction kit was utilized. Using 0.25µg random hexamer, 200units Superscript RNase H reverse transcriptase, as well as 2µg total RNA, 20µlfinal volume resulted. The reaction mixture had been incubated for fifty minutes at 50°C after being incubated for five minutes at 25°C. 3µL cDNA & 10µL of Applied Biosystems' SYBR Green Master Mix made up 20µL used for real-time PCR tests. The final forward and reverse primer concentrations were 300 pmol/L. They performed PCR cycle using Eppendorf Realplex apparatus (Eppendorf, Germany) & involved activating Taq polymerase for 10 minutes at 95 degrees Celsius and then denaturing template DNA for 15 seconds at the same point. 30 seconds were spent integrating the reading at 60°Celsius. 40 cycles of denaturation and annealing had been performed in order to ascertain Ct values for each gene that was subject of investigation. β-actin has been employed as an endogenous control, andmRNA expression fold change has been determined employing comparative Ct method and relative gene-expression quantification methods.

Primer

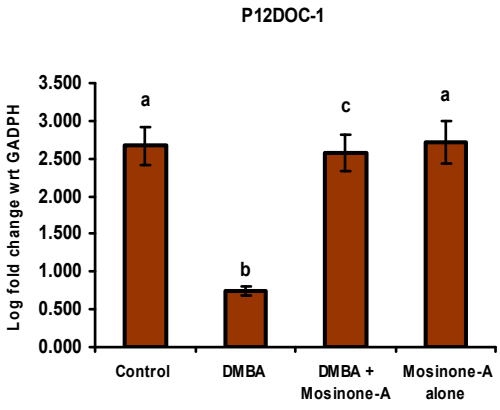
$P^{12doc-1}$	Sequence (5'->3')
Forward primer	AACGGAACGGAATGCCAGATCCTA
Reverse primer	TTCAGAGCCAAGTGAACCATGGGA
$P16^{INK4A}$	Sequence (5'->3')
Forward primer	TTCATGTGGGCATTTCTTGCGAGC
Reverse primer	CTGCCATTTGCTAGCAGTGTGACT
$p^{15INK4b}$	Sequence (5'->3')
Forward primer	TCCCAGATGAGGACAATGAGGCAA
Reverse primer	TTTGGAGGACAAGCTGGTGCAATG
GAPDH	Sequence (5'->3')
Forward primer	ATGACCACAGTCCATGCCAT
Reverse primer	TTGAAGTCAGAGGAGACCAC

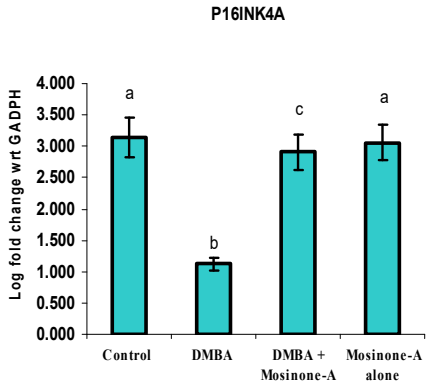
Statistical analysis

Shown values represent mean±SD (standard deviation). One-way ANOVA (analysis of variance) along with DMRT (Duncan's Multiple Range Test) have been employed for statistical comparisons. If p-value had been <0.05, values are considered statistically significant.

RESULT

The buccal pouch mRNA expression levels for p12DOC-1, p15INK4A, as well as p16INK4B in each control group as well as Animals used in experiments have been displayed quantitatively by RT-PCR in Figure 1. DMBA-treated hamsters (Group 2) showed increased expression of p12DOC-1, p15INK4A, & p16INK4B. But when DMBA-treated hamsters (Group3) were given Mosinone-A orally, expression of these genes was markedly elevated. Between group treated with Mosinone-A alone and the control group, no discernible changes in mRNA expression were found.





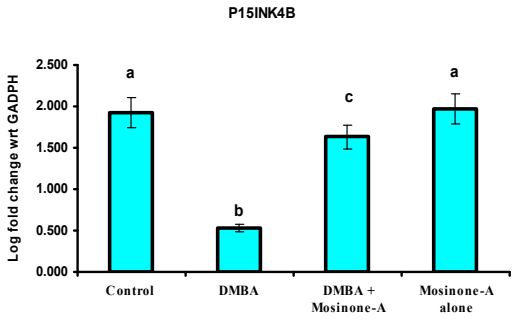


Figure 1: Shows the effect of Mosinone-A on the mRNA expression of (A) $p^{12doc-1}$, · (B) $p16^{INK4A}$ and (C)$p^{15INK4B}$,expression in control and experimental animals in each group by real time PCR studies. The results were expressed as log fold change in expression ratio between the groups. GADPH was used as an internal control. Values are expressed as mean ± SD for six animals in each group. Values not sharing a common superscript letter differ significantly at<0.05(DMRT)

DISCUSSION

Many chemotherapeutic compounds included in food components consumed by humans have antimutagenic and anticarcinogenic properties [12] are resistant to the proliferative or anti-apoptotic proteins that suppress cell proliferation in the development of cancer of the oral cavity **[13]**. The quick emergence of cellular signaling cascades that regulate gene expression might offer foundation for creating chemopreventive tactics that use occurring naturally substances for different types of cancer **[14]**. In this study, we have looked at the molecular evidence supporting Mosinone-A's chemopreventive effectiveness in DMBA-induced buccal pouch carcinogenesis.

Mosinone-A's varied pharmacological characteristics make it a promising chemopreventive drug, according to extensive research on chemoprevention of human pancreatic as well as prostate cancer cell lines. It has been noted that Mosinone-A contributes to induction of cellular differentiation, death, cell proliferation suppression, and cell cycle progression modification **[15]**. Additionally, it has been proposed that Mosinone-A's chemopreventive properties stopped hamsters' development of buccal pouch carcinogenesis brought on by DMBA. Known as important molecular markers for studies on cancer prevention, p12DOC-1, p16INK4A, and p15INK4B are among several factors that regulate cell survival and cell death by apoptosis. reduced expression of p12DOC-1, p16INK4A, & p15INK4B in the development of buccal pouch cancer.

At chromosome 12q24, highly conserved cellular gene P12DOC-1 is found. According to Todd *et al*., malignant keratinocytes and oral cancer tissue have been shown to exhibit consistently downregulated or deleted DOC-1 expression **[16]**. Our findings demonstrate a

decrease in p12DOC-1mRNA expression in hamster buccal pouch carcinogenesis triggered by DMBA.

Chromosome 9p21 contains the p15INK4B and p16INK4A gene cluster **[17]**. Although p15ink4B and p16INK4A are closely related and have comparable functions, p15ink4B functions as a non-reductant cell cycle checkpoint. Originating from extracellular cues such as transforming factor β and IFN-α, it functions as intermediary in route regulating cell cycle. p16INK4A can be activated, and p15INK4B can desensitize cell to these extracellular cues, which can lead to development of cancer **[18]**. p15INK4A, p16INK4B expression has been discovered to be negative predictive sign of cancer studies and has been reported to be missing or reduced in human oral malignancies. According to Reed *et al*., p16INK4A and p15INK4B expression was totally absent in more than 80% of oral squamous cell carcinomas **[19, 20]**. We found that DMBA-induced p12DOC-1, p15INK4B, and p16INK4A were downregulated.

CONCLUSION

As revealed by the upregulation of p12DOC-1, p15INK4B, and p16INK4A in mRNA expression in DMBA-induced oral carcinogenesis, the current study's findings unequivocally showed how mosinone-A suppresses cell growth, initiates differentiation as well as apoptosis, along with inhibits the development of tumors.

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