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Preventive Effect of Mosinone-A on Immunhistochemical Expression of VEGF Marker during 7, 12-dimethylbenz (a) Anthracene Induced Hamster Buccal Pouch Carcinogenesis

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Abstract: *Aim: The present study was investigated to evaluate immunohistochemical expression studies in DMBA induced hamster buccal pouch carcinogenesis.Materials and methods: A total number of 40 golden Syrian hamsters were randomized into 4 groups of 10 animals in each. Group I animals were served as untreated control. Groups II and III animals were painted with 0.5% DMBA in liquid paraffin three times per week for 14 weeks on the left buccal pouches. Group III animals were orally administered with Mosinone-A (2 mg kg^{-1}b.wt) starting one week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the sacrification of the animals. Group IV animals were received Mosinone-A alone throughout the experimental period. Results: Topical application of DMBA for 14 weeks induced buccal pouch carcinomas associated with increased expression of, VEGF, Oral administration of Mosinone-A significantly inhibited the development of HBP carcinomas as revealed by decreased expression of, VEGF, Conclusion: The result of the present study indicates that Mosinone-A can exerts protective effects against DMBA induced buccal pouch carcinogenesis. These findings suggest that Mosinone-A exerts its anticancer properties by inhibiting cell proliferation and inducing differentiation and apoptosis.*

Keywords*: Oral cancer; Mosinone-A; DMBA, VEGF,*

INTRODUCTION

Oral squamous cell carcinomas are the most frequent malignancy of the oral cavity, representing 90–95% malignancies in the oral region [1]. The development of oral carcinoma proceeds through a series of genetic changes involving the activation of oncogenes and loss of tumor suppressor genes. During this process, a disturbance in cell proliferation, dysregulation of cellular differentiation, insufficient apoptosis and genomic instability [2].

The golden Syrian hamster buccal pouch is an excellent target organ for studying oral carcinogenesis which, under the induction of 7, 12-dimethylbenz[a]anthracene consistently produces squamous cell carcinoma [3].

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DMBA, a potent organ and site specific carcinogen is commonly used to induce buccal pouch carcinogenesis in hamsters, Dihydrodiol epoxide, the ultimate carcinogen DMBA, mediate the carcinogenic process by inducing chronic inflammation through the over production of reactive oxygen species (ROS) [4]. The present study was designed to determine the antiproliferative potential of Mosinone-A in hamster buccal pouch carcinogenesis. Weanalyzed the expression of proteins related to angiogenesis such as (VEGF).

VEGF is a signal protein which plays essential roles in vasculogenesis and angiogenesis and it is well established that the formation of new blood vessels, is necessary for the growth and metastatic spread of solid tumors including oral carcinoma [5].

Mosinone-A is one of the novel mono-tetrahydrofuran ring acetogenin, from the bark of *Annonasquamosa*, viewing cytotoxic selectivities for the human pancreatic carcinoma cell line [6]. However, the mechanism by which Mosinone-A exerts its cytotoxic effect on oral cancer cells are not well understood. Therefore, we undertook this study to investigate the apoptotic and angiogenic associated proteins during the DMBA induced hamster buccal pouch carcinogenesis.

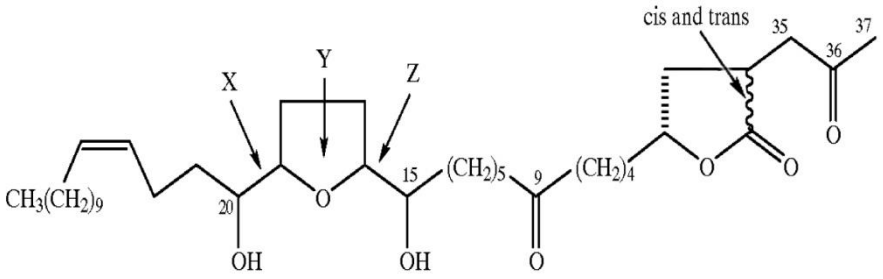

Figure 1: Structure of Mosinone-A ($C_{37}H_{64}O_7$)

MATERIAL AND METHODS

Animals

Eight to ten weeks old male golden Syrian hamsters, weighing 80-120g were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in the Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed in polypropylene cages and provided with a standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12h light /dark cycle.

Chemicals

The carcinogen, 7, 12-dimethylbenz[a]anthracene (DMBA) was obtained from Sigma-Aldrich Chemical Pvt. Ltd. Bangalore, India. All other chemicals used were of analytical grade, marketed by Himedia laboratories, Bangalore and Sisco Research Laboratories Pvt, Ltd, Mumbai, India.

Isolation of Mosinone-A

Mosinone-A was isolated from *Annonasquamosa* bark using the method of Maclaughlin [7]. The dried and pulverized bark of *Annonasquamosa* was extracted with ethanol. The residues were portioned between chloroform and water, and further portioned between 90% methanol and hexane to get hexane soluble residues. The hexane soluble residue was subjected into column chromatography over silica gel using hexane and chloroform followed by chloroform and methanol solvent system. The resulting fractions were combined on the basis of HPTLC analysis. Then, the combined fractions were run into column chromatography to get the final product of Mosinone-A, a whity waxy solid substance. The identity of isolated Mosinone-A was done by LC-MS and NMR. Its identity was confirmed by comparison to the reference Mosinone-A, which was purchased from Lock chemicals Ltd China. The yield and purity of the isolated Mosinone-A were found to be 0.21% and >90% respectively. For experimental studies Mosinone-A was first dissolved in 0.5% dimethyl sulfoxide (DMSO).

Experimental protocol

A total of 24 golden Syrian hamsters were randomized into 4 groups of 6 animals each. The first group served as the control group while the last 3 groups served as the experimental groups.

Group 1

Group 1animals were served as untreated control.

Groups 2

Groups 2 animals were painted with 0.5% DMBA in liquid paraffin three times per week for 14 weeks on the left buccal pouches (No:4 brush). Group 2 animals received no other treatment.

Group 3

Group 3 animals were orally administered with Mosinone-A (2 mg kg^{-1}b.wt) starting one week before the exposure to the carcinogen and continued on days alternate to DMBA painting, until the sacrification of the animals.

Group 4

Group 4 animals were received Mosinone-A alone throughout the experimental period.

Analytical procedure

The experiment was terminated at the end of 14^{th} week and all animals were sacrificed by cervical dislocation. The buccal pouch tissues were subdivided and variously processed for distribution to each experiment. Tissues were fixed in 10% formalin, embedded in paraffin and mounted on polylysinecoated glass slides. One section from each specimen was stained with haematoxylin and eosin. The remaining sections were used for immunohistochemical staining.

Immunohistochemistry

Immunohistochemistry is a technique in which antigens in tissue sections is detected by using antibodies and the detection is obtained by visualization of the antigen in a light microscopy according to the method of Kalhor*et al.,* [8]. Paraffin embedded tissue sections of 4µm thick are used for immunohistochemical analysis. The mounted paraffin embedded slices are deparaffinized in xylene and rehydrated with 100%, 90%, 80% and 70% ethanol followed by rising the tissues with PBS slides are placed in antigen retrieval buffer for 15 minutes and then allowed to cool to room temp for 20 minutes. The sections are then rinsed with 0.5 % BSA in PBS for 20minutes and treated with 5% normal horse serum and 1% BSA in PBS for 2hrs at room temp. The sections are then incubated overnight with primary antibodies at room temperature. All primary antibodies are diluted (1:100) after overnight incubation. The sections are washed in PBS. The sections are rinsed with 0.5% BSA in PBS thrice, 5minutes in each. The sections are then incubated with secondary antibodies which were diluted with 1% BSA in PBS for 2hrs. The sections are then observed under the microscope and photographs were taken.

RESULTS

The effect of Mosinone-A on VEGF, expression in the buccal pouch mucosa of control and experimental animals in each group. Tumors were considered positive when more than 10% of the tumor cells were stained. In DMBA painted hamsters, the mean protein expression of VEGF were significantly higher compared to control animals (Group 4). Oral administration of Mosinone-A to DMBA treated hamsters, (Group 3) significantly decreased expression ofVEGF compared to control animals. No significant changes were found in the expression of, VEGF, in control and Mosinone-A alone administered animals. Representative photomicrographs of immunostaining are shown in figures 1

STATISTICAL ANALYSIS

The percentage of positive cells in immunohistchemical was scored according to the method of Nakagawa et al. [9] as follows: $^{+++}$ =strong staining, more than 50% of cells were stained; $^{++}$=moderate staining, between 20 and 50% of cells were stained;$^{+}$ =week staining, between 1 and 20% of cells were stained; 0=negative, less than 1% of cell staining. The statistical data were analyzed by using the number of positively stained cells using Chi-square ($\chi 2$) test. The results were considered statistically significant if the p values were less than 0.05.

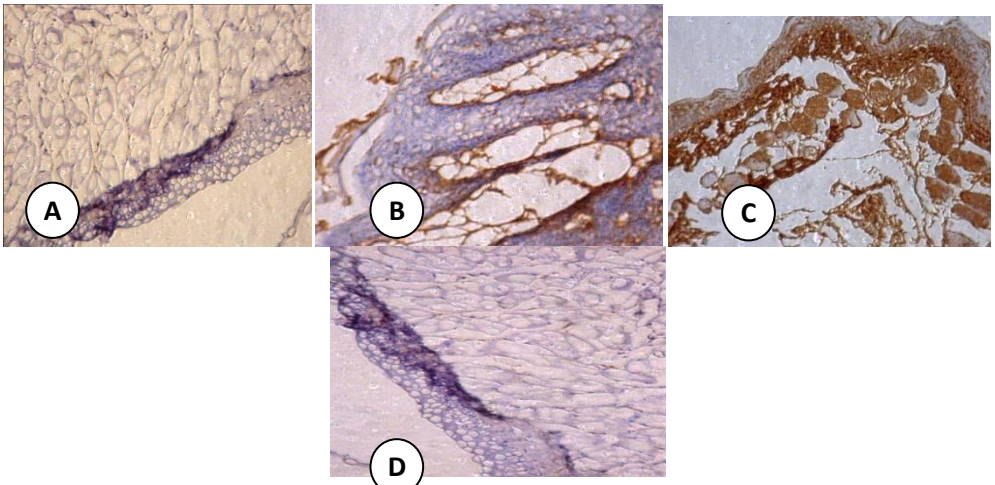

Figure 2:The VEGF expression of control and experimental animals in each group. (*A & D): Shows the Normal expression of VEGF in control and Mosinone-A alone administered animals. (B): Shows over expression of VEGF in DMBA alone treated animals. (C): Shows the decreased expression of VEGE in DMBA+Mosinone-A administered animals. Magnification= 20X*

DISCUSSION

In the present study, we have examined the molecular evidence to prove chemopreventive efficacy of the Mosinone-A in DMBA induced buccal pouch carcinogenesis A number of chemotherapeutic drugs are resistant to cancer treatment, passibly through the modulation of survival cell components such as proliferative or anti apoptotic proteins. [10].

Chemoprevention is a novel and promising approach to control, inhibit or suppress tumor formation using natural or synthetic entities [11]. A large number of dietary constituents ingest in the human diet exhibit anticarcinogenic and antimutagenic effects [12]. Mosinone-A is known to inhibit proliferation and induce cell cycle arrest in human pancreatic and prostate carcinoma cell line offers Mosinone-A as chemopreventive agent due to its diverse pharmacological properties. It has been pointed out Mosinone-A has a role to play in the initiation of cellular differentiation, apoptosis, and inhibition of cell proliferation and modification of cell cycle progression [13]. In DMBA painted hamsters, the protein expression of VEGF was significantly higher and Cytokeratin expression was significantly lower compared to control animals. Oral administration of Mosinone-A significantly decreased expression of VEGF compared to control animals. No significant changes of these markers control and Mosinone-A alone administerd animals.

VEGF plays a pivotal role in the regulation of normal and pathological angiogenesis, and it also increases vessel permeability and enhances endothelial cell growth, proliferation, migration and differentiation [14]. Several reports have suggested that over expression of VEGF in oral carcinogenesis [15]. In our present result have suggested that abnormal expression of VEGF in hamster buccal pouch carcinogenesis. Oral administration of Mosinone-A to DMBA treated hamster significantly prevented the abnormal expression of VEGF in DMBA treated hamsters.

CONCLUSION

The results of the present study clearly demonstrate that Mosinone-A acts as a suppressing agents by inhibiting angiogenesis and preventive tumourigenesis as exposed by down regulation of VEGF in DMBA induced oral carcinogenesis.

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Consent

It is not applicable.

Ethical Approval

"All authors hereby declare that "Principles of laboratory animal care" (NIH) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

Competing Interests

Authors have declared that no competing interests exist.

REFERENCES

[1] Kupferman ME, Myers JN. Molecular Biology of oral cavity squamous cell carcinoma. OtolaryngolClin North Am. 2006; 39:229–247.

[2] Kornberg LJ, Villaret D, Popp M et al. Gene expression profiling in squamous cell carcinoma of the oral cavity shows abnormalities in several signaling pathways. Laryngoscope. 2005;115: 690-698.

[3] Gimenez-Conti IB, Slaga TJ. The hamster cheek pouch carcinogenesis Model. J Cell Biochem1993; 17:83-90.

[4] Jerina DM, Yagi H, Lehr RE, et al. The bay-region theory of carcinogenesis by polycyclic aromatic hydrocarbons.In: Polycyclic aromatic hydrocarbons and cancer. Environment, chemistry and metabolism.New York : Academic Press; 1978.p.173-188.

[5] Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nat Med. 1995; 1:27-31.

[6] Hopp DC et al. Novel mono tetra hydro furan ring acetogenins from the bark of Annonasquamosa, showing cytotoxic selectivities for the human pancreatic carcinoma cell line PAC-2. J Nat prod. 1997; 60:581-586.

[7] Maclaughlin JL, Hoop DC. Selectivity cytotoxic acetogenin compound. United States patent US6242483. 2004;242-423.

[8] Kalhor N, Ramirez PT, Deavers MT, Malpica A, Silva EG. Immunohistochemical studies of trophoblastic tumors. Am J SurgPathol. 2009; 33:633-38.

[9] Nakagawa K. Yamamura K. Maeda S. et al. Bcl- 2 expression in epidermal keratinocytic diseases. Cancer. 1994;74: 1720-724.

[10] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell. 1997; 91: 479-89.

[11] Wilkinson J 4th, Clapper ML. Detoxification enzymes and chemoprevention. ProcSocExpBiol Med. 1997; 216:192-200.

[12] Ragers AE, Zeisel SH and Groopman J. Diet and carcinogenesis. Carcinogenesis. 1993; 14:2205–2217

[13] Zeng L. Novel mono tetra hydro furan ring acetogenins from the bark of *Annonasquamosa,* showing cytotoxic selectivities for the certain specific human tumor cell line PAC-2. J Nat prods. 1996; 59:1035-1042.

[14] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003; 9:669-76.

[15] Sappayatosok K , Maneerat Y. Swasdison S.et al. Expression of pro-inflammatory protein, iNOS, VEGF and COX-2 in oral squamous cell carcinoma (OSCC), relationship with angiogenesis and their clinico-pathological correlation. Med Oral. Patol. Oral. Cir. Buccal. 2009; 14: 319-324.