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Chemotherapeutic and chemopreventive effect of ZnO nanoparticles on DMBA/croton oil induced mice skin carcinogenesis

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ABSTRACT

The present study was conducted to evaluate the antitumor effect of cationic ZnO nanoparticles (NPs) on 7,12–dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in male Swiss albino mice. The effect of ZnO(NPs) on tumor growth was studied by following parameters: The tumor incidence, tumor yield, tumor burden and cumulative number of papillomas, average latent period and tumor inhibition multiplicity. DMBA was applied on the shaved back of mice for the induction of tumor and left for two weeks, after that croton oil was applied thrice a week. In experimental mice ZnO was applied from croton oil application and continued upto next 14 weeks. The tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were found to be higher in the control (without ZnO treatment) as compared to experimental animals (ZnO treated). The differences in the values of the results of experimental groups were statistically analyzed and found to be significant in comparison to the control group. The latency period in treatment of experimental groups significantly increased as compared with the control group**.** The average weight and diameter of tumors recorded were also comparatively lower in the ZnO(NPs)-treated groups. Taken together, these findings indicate the chemopreventive and thearapeutic potential of ZnO (NPs).

Keywords: Antitumor, Cancer, DMBA, Tumors, ZnO nanoparticles

INTRODUCTION

Nanotechnology is a multidisciplinary field, in which principles of Chemistry, Biology and Engineering are used[1-7] Nanotechnology has been greatly appreciated as a potential tool for cancer treatment. Because most biological processes including those are cancer related occurs on nanoscale.[8]

Nanoparticle-assisted drug delivery, cell imaging, and cancer therapy are important biomedical applications of nanotechnology.[9-11] NPs, including metal oxides, are promising materials for applications in medicine.[12] The potential application of ZnO NPs in biomedical field includes antibacterial applications, bio sensing, and cancer diagnosis and therapy applications.[13,14] There are some useful properties of ZnO (NPs) for biomedical applications.

Cancer is not a single disease but is a group of many diseases that all share many biological and pathological characters.[15] Cancer is the second leading causes of death worldwide.[16] It has emerged as a life threatening non-communicable disease. Abnormal proliferation, invasiveness and metastasis of cells are main characters of cancer. It is a disease with high number of sufferers in the world. About 7.6 million people died from cancer in 2008 and about 12.4 million new cases are diagnosed each year.

Skin Cancer is the most common type of malignancy in the world. It has a very high rate of incidence exceeding the sum of other cancers combined.[17,18] The incidence of NMSC (non melanoma skin cancer) has dramatically increased during the last decade and at present it accounts for 30% of all cancers.[19,20] It is estimated that more than 8500 people in U.S. are diagnosed with skin cancer every day.[21] Melanoma is projected to be the fifth most common cancer for men and the seventh most common cancer for women in 2016.[22]

In the present study we want to use ZnO(NPs) as chemotherapeutic and chemopreventive agent for the treatment of skin cancer by topical application ,on the back of mice skin.

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For this study DMBA and croton oil were used for carcinogenesis. Following morphological parameters were studied to know the effect of ZnO(NPs) in this experient : the tumor incidence, tumor yield, tumor burden , cumulative number of papillomas, body weight, tumor size, tumor weight average latent period and tumor inhibition multiplicity.

MATERIALS AND METHODS

Animals

The study was conducted on 7-8 weeks old and body weight having 24 ± 2 g. Random breed male Swiss albino mice were kept in polypropylene cages, one mouse per cage, in the animal house under controlled conditions of temperature (25°C ± 2°C) and light (14 light:10 dark). These mice were fed a standard mouse feed procured from Aashirwad Industries, Chandigarh (India) and water *ad libitum*. The protocol of the experiment was approved by the Institutional Ethical Committee and animal care and handling was done according to the guidelines set by the World Health Organization, Geneva (Switzerland) and the Indian National Science Academy, New Delhi (India).

Chemicals

7, 12-Dimethyl Benz (a) anthracene (DMBA) and croton oil were procured from Sigma Chemical Co., USA. DMBA was dissolved at a concentration of 100 µg/ 100µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.ZnO 70 nm cationic nanoemulsion was bought from Sisco Research Limited (Maharashtra) India. Stearic acid was used of Himedia lab pvt ltd,Nashik.

Experimental design

Induction of tumor

Murine skin carcinogenesis is a stepwise process, consisting of initiation, promotion and progression.[23] For the induction of skin tumors, the dorsal skin of the animals in the back area was shaven 3 days before the commencement of the experiment and only those animals in the resting phase of hair cycle were chosen for the study, and 100 µL DMBA (100 µg/100 µL acetone) was applied. Two weeks after giving DMBA initiator the tumor promotion started by the topical application of 100 µL croton seed oil (1% v/v in acetone), thrice a week, for the next 14 weeks.

ZnO (NPs) were used as 0.5mg/cm^2 and 3% stearic acid was mixed in ZnO nanoemulsion to prevent the irritating effect of this nanoemulsion. Distilled water was add to make proper concentration.

During the experiment, all mice were observed daily and body weight was taken once in a week. Tumors appearing on the shaven area of the skin were recorded at weekly intervals in all of the above groups. Tumors that persisted at least for 2 weeks or with a diameter of more than 2 mm were taken into consideration for the final evaluation of the data.

Animals for this experimental study were divided into the following groups:

Group I: Untreated Mice

Animals of this group did not receive any treatment.

Group II: Vehicle treated Control

Animals of this group were given topical treatment by Acetone (100 µl/ mouse) on the shaven dorsal skin and double distilled water (100 µl/ mouse/ day) by oral route, for 16 weeks.

Group III: Carcinogen treated Control (Positive Control)

In this group of mice DMBA was applied topically over the shaven area of the skin with a single dose of 100 µg of DMBA in 100 µl of acetone. After two weeks later of DMBA application Croton oil (100 µl of 1% croton oil in acetone) was applied three times per week, until the end of the experiment (i.e. 16 weeks).

Group IV: ZnO treated Experimental-1: Animals of this group were given DMBA and Croton oil as given in the group III .These animals were topically treated with ZnO NPs, one hour before the croton oil application, starting from Croton oil application to the end of the experiment.

Group V: ZnO treated Experimental-2: These animals were treated same as the Group IV except ZnO NPs was applied one hour after croton oil application.

Morphological study

1 Cumulative number of tumors

Till the terminations of the experiment the total number of tumors appeared, were recorded.

2 Tumor incidences

The number of mice carrying at least one tumor was expressed as percent incidence.

3 Tumor yield

The average number of tumors per mouse was calculated.

4 Tumor burden

The average number of tumors per tumor-having mouse was calculated.

5 Tumor Diameter

At the time of sacrifice the diameter of each tumor was measured.

6 Tumor Weight

The weight of each tumor was recorded at the termination of experiment.

7 Body weight

The weight of each mouse was recorded once in a week and before sacrificing it.

8 Average latent period

The time lag between the application of the promoting agent and the appearance of 50% of tumors was determined. The average latent period was calculated by multiplying the number of tumors appearing each week by the time in weeks after the application of the croton oil, and dividing the sum by the total number of tumors.

Average latent period = $\sum FX / N$

Here F is the number of tumors appearing each week, X is the number of weeks, and N is the total number of tumors.

9 Inhibition of tumor multiplicity

Total number of tumors in carcinogen treated control—Total number of tumors in ZnO NPs treated group X100 / Total number of tumors in carcinogen treated control.

RESULTS

Morphological study

As shown in Table, treatment with the ZnO NPs influenced the various stages of skin carcinogenesis in mice. In Group I, the body weight gradually increased in the experimental period, but body weight decreased in the carcinogen-treated control animals.

Table 1: Showing chemopreventive effect of ZnO(NPs) application on skin carcinogenesis.

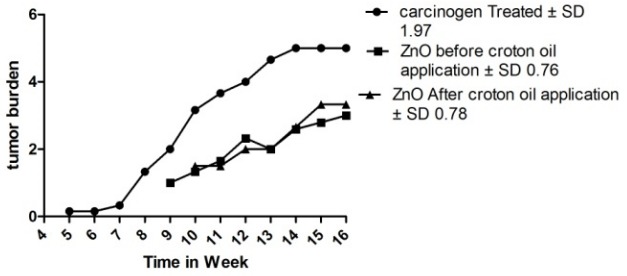
Treated groups	Body weight		No. of Tumors	Tumor weight
	Initial	Final		
l (Normal)	25.47± 5	35.74±6.60		-
ll (Vehicle treated control	26.83±0.87	34.66±0.93		-
Ll+-*9/887`l (Carcinogen treated control)	23±1.08	22.08±1.02	30	1.15±0.09
lv (Experimental 1) Before	22.5± 0.42	26.83±0.4	16	0.44±0.004
v (Experimental 2) After	23.66±0.42	30±0.50	10	0.32±0.004

Data are presented as mean ± SD

Table 2: Chemopreventive effect of ZnO (NPs) on Chemical-induced Skin Carcinogenesis .

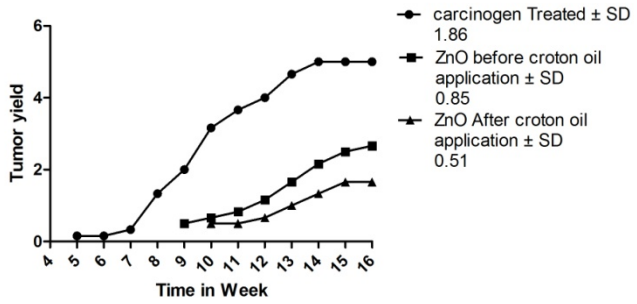
Treated groups	Tumor yield	Tumor burden	TIM*	ALP*	Tumor incidence (%)
l (Normal)					
ll (Vehicle treated control					
lll (Carcinogen treated control)	5. ± 0.44	6 ± 0.42		9.4	100
lv (Experimental 1) Before	2.66± 0.28	3.2±0.25	46.66	10.56	83.33
v (Experimental 2) After	1.66± 0.19	3.33± 0.29	66.66	11.4	50

Data are presented as mean ± SD. TIM (Tumor inhibition multiplicity); ALP (Average latent period)



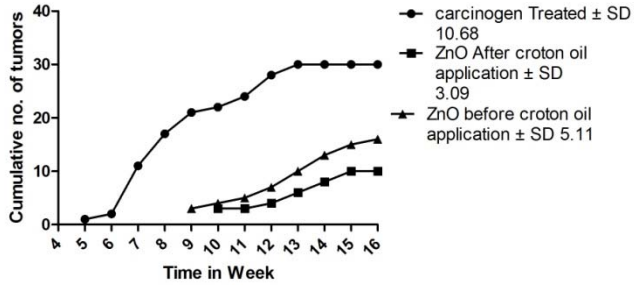
Graph 1: Graph showing tumor burden per week

As this graph represents the effect of ZnO(NPs) on tumor burden. By comparative study we found that in carcinogen treated mice tumor started to appear from 5th week and tumor burden was 6 ± 0.42. In mice who got ZnO(NPs) treatment before croton oil application tumor burden was reorded 3.2±0.25 and there was no tumor burden before 9th week. In mice who got ZnO (NPs) treatment after croton oil application revealed the tumor burden from 10th week and average tumor burden was found 3.33± 0.29.



Graph 2: Graph showing tumor yield per week

This graphical presentation shows that tumor yield was 5. ± 0.44, 2.66± 0.28 and 1.66± 0.19 in carcinogen treated mice, mice who got ZnO(NPs) treatment before croton oil application and in mice where ZnO(NPs) was used as chemotherapeutic agent, respectively.



Graph 3: Graph showing cumulative number of tumors per week

This graph shows that cumulative number of tumors were 30, 16 and 10 in carcinogen treated mice, mice whose back was applied with ZnO(NPs) before croton oil application and mice who got ZnO(NPs) treatment after croton oil application, respectively. This graph also represents that in carcinogen treated control mice tumors started to appear very early, from 5th week but when ZnO(NPs) used as chemotherapeutic and chemopreventive agent tumors started to appear from 9th and 10th week, respectively.

DISCUSSION

Topical treatments of skin disease are preferred because it can be applied directly to the problem area and there is lower risk of systemic side effects.[24] Low toxicity, biocompatibility and biodegrability of ZnO makes it a material of choice for biomedicine.[25]

In mice skin carcinogenesis model DMBA, a polycyclic aromatic hydrocarbon is used as cancer initiator and croton oil is used as promoter. The tumor promoting potential of croton oil is due to TPA, a phorbol ester present in it as a major constituent. Skin carcinogenesis is a multistep process, consisting of inhibition, promotion, and progression.[26] DMBA-DNA stable formation can lead to the induction of mutation by

activating proto-oncogen or inactivating tumor suppressor genes, which is an important event during tumor initiation. DMBA requires metabolic activation to deploy its carcinogenicity.[27] It is metabolised by, the enzyme of CYP450, CYP1A1 and CYP1B1 to the ultimate carcinogen 1, 2-epoxide-3, 4-diol DMBA. This epoxide complex with DNA and leads to mutation, which is prerequisite for the development of tumor. Treatment with TPA has been exhibited to induce a variety of changes in murine skin which includes dark basal keratinocytes and sustained epidermal hyperplasia, reactive oxygen species formation in epidermis, elevated epidermal cyclooxygenase.[28] Nanotechnology is definitely a medical boon for diagnosis, treatment and prevention of various diseases including cancer. It supports and expand the scientific advances and builds on our understanding of the molecular underpinning of cancer and its treatment. In these days, ZnO NPs have received much attention for their implications in cancer therapy.

The present study revealed the therapeutic property of ZnO(NPs) in group V. It can be exhibited by some of these explainations. Overexpressed cytochrome C level by ROS can lead to cancer cell death. To mimic the natural killing system, as in several carcinomas activated neutrophils exert anti-tumor effect by the increased production of ROS and possibly due to higher level of oxidants in cancer cells. Recent developments in cancer research show that most of apoptotic stimuli share common mechanistic pathways that is the generation of ROS through oxidative stress. ROS typically include the superoxide radical(O2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (-OH) which damages biomolecules such as lipids, DNA and proteins and eventually leads to cell death. It was found that the basis for the selective effect of the ZnO(NPs) on cancer cells may be due to the increased generation of ROS and to an increased sensitivity of these cells to oxidative stress. It has been described that following three levels of ROS oxidative stress have been observed for ZnO(NPs) in immortalized phagocytic or bronchial epithelial cells leading to death by either necrosis or apoptosis. Tier 1 involves increase in antioxidant enzymes, Tier 2 includes an increase in potent pro-inflammatory cytokines leading to inflammation, and while Tier 3 is characterized by mitochondrial disturbance.Preferantial cytotoxicity also appears to be related to the proliferative capacity of the cell. Because when normal non dividing cells are stimulated to proliferate, demonstrate an increased sensitivity to ZnO (NPs) induced death. This inherent differential toxicity of ZnO (NPs) against rapidly dividing cancer cells shows their potential use as anticancer agents. There are some evidences that show that primary mechanism of ZnO nanoparticle cytotoxicity might proceed by inducing the generation of ROS, which acts as critical signaling molecules to start apoptosis.[29,30] Apoptosis is a major target of cancer treatment. Caspase-3 enzyme is capable for irreversible apoptosis

ZnO (NPs) have shown the higher activity of Caspase-3. It has found that ZnO (NPs) induce apoptosis in cancer cells, mediated by ROS via p53, bax/bcl-2 and caspase pathway through which most of the anticancer drugs triggers apoptosis.

ZnO (NPs) showed to exhibit strong protein adsorption properties, which is useful to modulate cytotoxicity, metabolism or other cellular responses. Studies demonstrated that the cytotoxic properties of ZnO(NPs) against cancerous cells is directly related to size.

In the present study we used cationic 70 nm ZnO nanoemulsion. This ultra-small size is comparable to naturally occurring proteins and biomolecules in the cell. This size of ZnO(NPs) can facilitate their entry into tumor tissues, and their subsequent retention by a process recognized as the enhanced permeation and retention effect (EPR). The EPR phenomena is a combination of “leaky” tumor blood vessels due to alterations in angiogenic regulators, enlargement in gap junctions between endothelial cells and compromised lymphatic drainage in the tumor microenviroment. Electrostatic interaction between positively charged nanomaterials and cancer cells, frequently having a high concentration of anionic phospholipids on their outer leaflet and large membrane potential is also an important consideration for cell uptake .

Cationic surfactant coating is helpful in rendring positive charge to NPs therefore improving their interaction with cells and tissues. Cationic surfactant coating is important in size reduction. Thus surface charge of cationic NPs is effective for cellular uptake.

This study also demonstrated that ZnO (NPs) have chemopreventive properties, towards skin carcinogenesis in mice. Here topically applied ZnO(NPs) might remove potentially damaging toxins called free radicals or it could reverse free radical damage caused by croton oil application and decreased the number of gene errors (mutations) that can lead to cancer. It is also possible that ZnO(NPs) worked here as cyclooxygenase inhibitor which is required for the synthesis of prostaglandin E2, that stimulates the proliferation of cells.

Significance

The ultimate goal of cancer therapeutics is to increase the survival time and the quality of life of the patient. Recent research on nanoparticles has promising results to achieve this goal. Despite the recent advancement in the therapeutic, significant challenges still present in the field of skin cancer. Squamous cell carcinoma can be treated with radiotherapy, chemotherapy and mohs surgery but these treatment procedures may kill healthy cell and cause toxicity. Topical delivery is particularly attractive for the therapy of skin cancer. Topical administration of anticancer drugs is an alternative for reducing side effects and for increasing drug targeting and therapeutic benefits. ZnO nanoparticles can preferentially kill cancer cells without impacting normal cells, a research that can potentially treat the cancer without the side effects caused by chemothearapy. While posing no side effects on normal cells the marked difference between cancer cells and normal cells suggests an exciting potential for ZnO nanoparticles as normal alternative to skin cancer therapy. 5-FU when applied directly on the skin, it kills tumor cells near the skin's surface, but cannot reach cancer cells that may have grown deeply into skin for this reason treatment with 5-FU generally is used only for pre cancerous conditions such as actinic keratosis (AKs) and for

some very superficial skin cancers. The use of ZnO nanoparticles may be better in this regard for other types of skin cancers.

CONCLUSION

In conclusion, the present study shows that there is reduction in total number of tumors, decrease in tumor yield and tumor burden, in both the ZnO (NPs) treated groups. Average latent period, tumor inhibition multiplicity and body weight was increased in ZnO treated groups. Percent incidence of tumor is also decreasing in both the treated groups. Overall this finding is a good evidence to show the anticancer effect of ZnO nanoparticles. This study also shows that ZnO(NPs) is better therapeutic than chemopreventive for cancer treatment. By this study we can say that ZnO(NPs) may be used as an anticancer agent.

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