IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 07, pp. 2636-2640, July, 2016

Research Article

CHEMOPREVENTION OF DMBA INDUCED SKIN CARCINOGENESIS IN SWISS *ALBINO* MICE BY QUININE SULFATE

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ARTICLE INFO

Article History:

Received 15th April 2016
Received in revised form
24th May 2016
Accepted 19th June 2016
Published online 31st July 2016

Keywords:

7, 12-Dimethylbenz (a) Anthracene,
Skin Cancer,
Tumor,
Quinine Sulfate,
Oral Administration.

ABSTRACT

7, 12-dimethylbenz (a) anthracene induced and croton oil promoted mouse skin carcinogenesis is a useful murine model to show genetic and biological changes involved in tumor promotion, and also for selecting of new cancer protective agents, because this model displays a preneoplastic condition during carcinogenesis in the form of papillomas, which are easily visible. The aim of this study is to show the effect of quinine sulfate against skin cancer in Swiss *albino* mice (Mus_musculus).Oral administration of the Quinine sulfate at the dose rate of 12 mg/kg body weight showed a significant reduction in the tumor burden, tumorweight, tumor size and cumulative number of tumors. Moreover there was decrease in the number of tumor bearing mice and their onset of tumors. These findings suggests the protective effect of Quinine sulfate in skin cancer but a little higher dose 15 mg/kg body weight did not show protective effect.

INTRODUCTION

Skin is the protective barrier to protect from heat or cold, chemicals, UV-radiation and bacteria. Skin cancer is the most common of all cancer types, and its incidence is increasing rapidly all over the world. Skin cancer contributes approximately 30% of all newly diagnosed cancer in the world, resulting from repeated sunlight exposure in which ultraviolet B (UVB) radiation is a major environmental carcinogen that induces nonmelanoma skin cancer (Mallikarjuna *et al*. 2004; Soehnge *et al.* 1997; Kraemer, 1997). Cancer is one of the most important causes of death. It is the second leading cause of death in developed countries after cardiovascular diseases (Parkin *et al*. 2001). Due to the increasing rise in incidence of skin cancer patients throughout the world development of novel methods to prevent skin cancer exhibits an important goal (Greenleee *et al*. 2001; Gupta and Mukhtar, 2002). Chemoprevention is a method of cancer control, and is a realistic approach by which the occurrence of the disease can be entirely prevented by the use of one or more naturally occurring and / or synthetic compounds (Lamson and Brignall, 2001; Ley and Reeve, 1997).

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It has enormous potential to prevent, suppresses, inhibit or reverse initiation and progression phases of carcinogenesis (Goldsmith, 2001; Manoharan *et al*. 2009). DMBA is a ubiquitous environmental pollutant which has high carcinogenic potential and it is considered one of the etiological factors for human cancers because it is present in cigarette smoke and environmental mixtures. The effect of tumor incidence byQuinine sulfate was evaluated on two-stage skin carcinogenesis, induced by a single application of DMBA (initiator) and two weeks later promoted by the repeated application of croton oil (promoter) thrice per week, for the period of 16 weeks.

MATERIALS AND METHODS

Chemicals

In this study 7, 12-dimethyl benz (a) anthracene (DMBA) was used as the initiator and the croton oil served as promoter. Both of these chemicals were procured from Sigma Chemicals Co. (St Louis, MO, USA). DMBA was dissolved in Acetone at a concentration of 100 µg/100 µL. Croton oil was mixed in acetone to give a solution of 1% dilution.

Animals

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). The present experiment was conducted on Swiss albino mice 7–8-week old and weighing 24 ± 2 g. which were selected from a inbreed colony. The animals were kept on standard synthetic pellet diet (procured from Aashirwad Industries, Chandigarh, India) and water *adlibitum*. These animals were kept in polypropylene cages in the animal home facility under controlled conditions of temperature (25 ± 2°C) and light (14 light:10 dark).

Experimental design

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

Group I: Normal Mice

Animals of this group did not receive any treatment.

Group II: Vehicle treated Control

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

Group III: Carcinogen treated Control (Positive Control)

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

Group IV: Oral Quinine sulfate treated, Experimental-1

Animals of this group were given DMBA and Croton oil as group III .These animals were given oral dose of 12 mg/kg body weight starting from Croton oil application to the end of the experiment.

Group V: Oral Quinine sulfate treated, Experimental-2

These animals were treated same as Group IV except change in oral dose which was 15 mg /kg body weight.

Induction of tumor

Murine skin carcinogenesis is a stepwise process, consisting of initiation, promotion and progression.For the induction of skin tumors, dorsal hairs between the cervical and caudal portions of the animals were removed before 2 days to the initiation of the experiment, 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), three times in a week, for the next 14 weeks. During the experimentation of 16 weeks, all mice were observed daily and body weight was taken weekly. Tumors appearing on the shaven area of the skin were recorded at weekly intervals in all of the above groups. Only those 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. Skin tumors which were not seen after one observation were not accounted.

Morphological study

1 Cumulative number of tumors

The total number of tumors appeared till the terminations of the experiment, 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-bearing mouse was calculated.

5 Tumor Diameters

The diameter of each tumor was measured at the end of experiment.

6 Tumor Weight

At the termination of experiment the weight of each tumor was recorded.

7 Body weight

The weight of each mouse was recorded once in week and before sacrifice.

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.

$$\sum FX / N$$

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 quinine sulfate treated groupX100 / Total number of tumors in carcinogen treated control

RESULTS

Morphological study

As shown in Table, treatment with the Quinine sulfate affected 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. Animals of Group III exhibited 100% tumor incidence after the treatment with DMBA/croton oil alone, while the animals of Groups I and II did not show any tumor appearance.

Table 1. Antitumorigenic Potential of QS* on DMBA-induced Skin Carcinogenesis in Mice

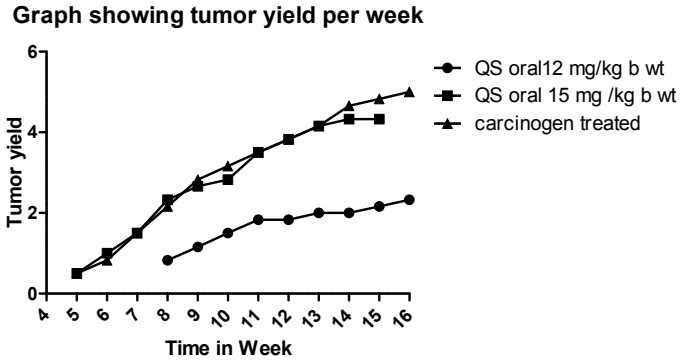
Treatment group	Body weight(gm)		No. of tumors	Tumor weight (gm)
	Initial	Final		
Normal	25.47 ± 5.15	35.74 ± 6.60	—	—
Negative control	26.83 ± 0.87	34.66 ± 0.93	—	—
Positive control	23 ± 1.08	22.83 ±1 .02	30	1.15 ± 0.09
12mg /kg/bw oral	26.83 ± 0.79	32.4 ± 0.68	14	0.39 ± 0.002
15mg/kg/bw oral	27.83 ± 1.16	25.5±1.35	26	0.803 ± 0.02

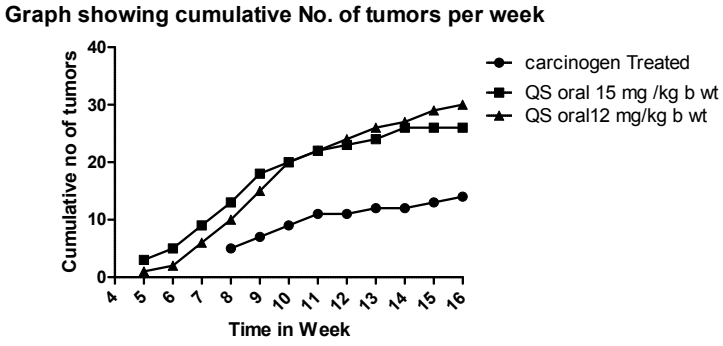
Data are presented as mean ± SE,* QS (Quinine sulfate

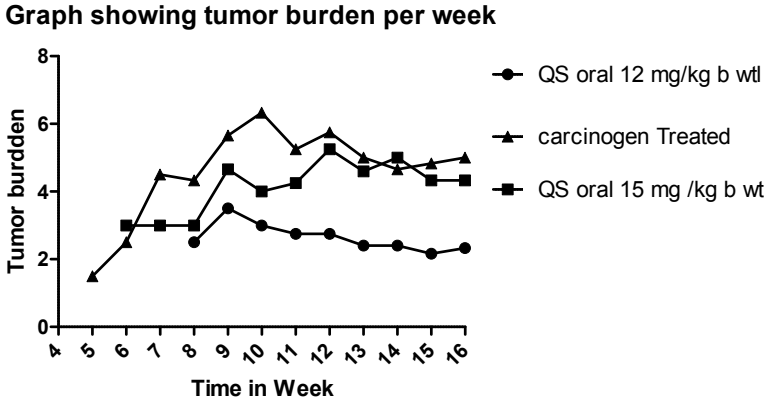
Table 2. Effect of Quinine Sulfate on Chemical-induced Skin Carcinogenesis in Mice

Treatment group	Tumor yield	Tumor burden	ALP*	TIM* (%)	Tumor incidence (%)
Normal	—	—	—	—	—
Negative control	—	—	—	—	—
Positive control	5. ± 0.44	6 ± 0.42	9.4	—	100
12mg/kg bw oral	2.33 ± 0.48	2.8 ± 0.15	11.71	53.33	83.33
15mg/kg bw oral	4.33 ± 0.41	5.2 ± 0.27	10.15	13.33	100

Data are presented as mean ± SE, TIM (Tumor inhibition multiplicity), ALP (Average latent period)







DISCUSSION

Quinine sulfate is rapidly and almost completely absorbed from the gastrointestine tract .It is 80% metabolized by the liver. The oral dose rate for children under 11 years oldis, 10 mg/kg /every 8 hours for 3,7or 10 days and for adults 600 mg after per 8 hours. Throughout the world Cancer is one of the foremost causes of morbidity and mortality (jaffe and lopez). Cancer is a group of more than 100 different diseases characterized by uncontrolled growth of cells, local invasion of tissues and metastases. Carcinogenesis is a multistep molecular process which is induced by genetic and epigenetic factors, during this interruption occurs in different processes like cell proliferation, apoptosis, differentiation and senescence pathway. DMBA a poly aromatic hydrocarbon (PAH), has a potent tumor initiating capacity, to become an ultimate carcinogen it undergoes metabolic activation (Manoharan *et al.*2012). It is metabolically activated in cells by cytochrome $_{p450}$ enzyme to reactive intermediates that damage DNA. Many enzymes concerned with DNA repair are destroyed by different type of cancer. These enzymes may be induced by DMBA in experimental animal models.12-O-tetradecanoylphorbol-13-acetate (TPA) increased formations of free radicals. Reactive oxygen species (ROS) play an important role in the pathogenesis of several chronic diseases including cancer (Li *et al.* 2013). The tumor promotion stage can be triggered by repeated application of croton oil, which is rich in phorbol esters, ultimately producing a squamous papilloma (Allen *et al.*2003; Slaga *et al. 1996*). DMBA as tumor initiator induces an irreversible and specific mutation in mouse skin by A to T transversion in codon of the Ha-ras gene in more than 90% cases (Balmain andBrown, 1988; Fujiki *et al.*1989).

The quinoline scaffold is present in many classes of biologically-active compounds (Tiwari *et al.*2000; Metwally *et al.* 2006; Kidwai *et al.* 2000). Some investigated compounds showed antineoplastic activity (El-Subbagh *et al.* 2000; Watson *et al.*2001). In recent years, large numbers of quinoline derivatives have been synthesized and their various significant biological activities, including different types of cancers, have been reported. When the cancer cell line (HEp-2) was treated with these products the result showed significant cytotoxic effect in all tested samples in comparison with the control compound. Compounds that contain the planar polycyclic quinoline core are widely used in the treatment of cancer. Most of these compounds exert their cytostatic effect through DNA intercalation, with further interaction with topoisomerasell.Cationic molecules have been shown to be the most effective DNA intercalators (Jerom andSpencer 1988) ,such as the quaternary or active nitrogen in the ring structure of quinoline containing compounds. Some fused tri and tetracyclic quinoline derivatives were found to have potent antitumor activity comparable to potent anticancer drugs like adriamycin, actinomycin and ellipticine which work by DNA intercalative property. Imiquimod is an IFN (interferon)-α inducing Quinoline derivative with antitumor effectiveness. Imiquimod cream uses immune system to attack cancers (Sterry *et al.* 2002; Bath-Hextall *et al.* 2004; Geisse *et al.* 2005; Gollnick *et al.* 2005; Schulze *et al.* 2005). This means it uses body's natural defenses to kill the skin cancer cells. It is thought that imiquimod induces cells to produce more interferon.

Imiquimod was a potent inhibitor of tumor growth in mice when administered orally at nontoxic doses. Other mechanisms may be involved in mediating the observed antitumor effects. For example, treatment with imiquimod reduced tumor-induced angiogenesis.Imiquimod proved to be highly potent in the inhibition of a wide range of murine tumors. Thus imiquimod may inhibit tumors induced by chemical carcinogens, as has been noted for IFN and IFN-inducing pyrimidinones. Imiquimod induces other cytokines which affect other aspects of the immune response. Topoisomerase is the target of several important classes of anticancer drugs, including the epiphyllotoxinepopside and the anthracyclin doxorubicin..Quinoline derivatives are widely used antibiotics that target DNA gyrase a bacterial form of topoisomerase II (Uchiyama *et al.*1998).These compounds exhibits good cytotoxic activity towards tumor cell lines. A novel quinoline derivative, TAS-103 was developed as an anticancer agent targeting topoisomerase, with marked efficacy in solid tumors. Cyclooxygenases (COX-2) are rate limiting enzymes in arachidinic acid metabolism and prostaglandin production.The topical diclofenac drug works as a nonspecific COX-2 inhibitor and is an effective and well tolerated treatment for actinic keratosis,which is a principal precursor of cutaneous SCC(squmaous cell carcinoma).Oral and topical COX-2 inhibitors have chemopreventiveactivity against skin cancer in animal models. Some 4 carbonyl quinoline derivatives and 2,3-diaryl quinoline derivatives possessing a methyl/sulfonyl COX-2 pharmacophore were evaluated as selective COX-2 inhibitors and were found more potent than the reference drug celecoxib.

3-[3-(7 chloro-quinolin-4-yl amino) phenyl]-1-(4methoxy-phenyl) prop-2-enone citrate (CITme) is a substituted quinoline derivative synthesized as a potential antitumor agent and it works by cytotoxic mechanism. The present study demonstrates that the papillomas initiated by DMBA and promoted by croton oil decrease in group iv (12mg/kg b wt), in comparison to carcinogen treated control group. Maximum tumor inhibition in this group may be due to inhibition of DMBA metabolism, delay in the promotion phase of carcinogenesis by down regulating production of reactive oxygen species or quinine sulfate may work here as other anticancer quinoline derivatives to decrease cumulative number of tumors, tumoryield, tumor incidence and tumor burden by inhibiting. COX-2 enzyme, working as Topoisomerase inhibitor, by inducing interferon-α, by DNA intercalative property or may exert some other cytotoxic mechanism. Thechemopreventive efficacy of Quinine sulfate in this study suggests its potential role as an anticancer agent.
As this study did not show considerable changes in group v hence it may be suggested that this dose rate of 15mg/kg b wt may be toxic or some enzymatic disturbance may occur at this dose rate. It can also be mitogenic at this dose. The mechanism of action of Quinoline sulfate at higher dose on skin carcinogenesis requires further study which is in progress. Cancer has a major impact on society across the world. Cancer statistics of last few years describe what is happening in large groups of people and provides a picture of the burden of cancer on society.The ultimate goal of cancer therapeutics is to increase the survival time and the quality of life of the patient. Despite the recent advancement in the therapeutic, significant challenges still present in the field of skin cancer. Recent research has promising results to achieve this goal.

This therapy may be especially useful for everyone because quinine sulfate is one of the least expensive available drugs as compared to standard drug, 5 fluorouracil (5 -FU), for skin cancer treatment, which is expensive so not easily available for everyone and it is used only for pre cancerous conditions such as actinic keratosis (AKs) and for some very superficial skin cancers.The use of Quinine sulfate may be better in this regard for other types of skin cancers.

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