Food and Chemical Toxicology 59 (2013) 687–695

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Skin tumorigenic potential of benzanthrone: Prevention by ascorbic acid

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ARTICLE INFO

Article history:
Received 23 April 2013
Accepted 6 July 2013
Available online 16 July 2013

Keywords:
Ascorbic acid
Benzanthrone
Tumorigenic potential
Skin allergy
Skin tumor

ABSTRACT

Benzanthrone (BA) exposed occupational workers have been found to exhibit toxicological manifestations in the skin, thus it is quite likely that long term exposure may lead to skin tumorigenicity. Thus, attempts were made to elucidate the tumor initiating and promoting potentials of pure (PBA) and commercial benzanthrone (CBA). Additionally, the preventive role of ascorbic acid (AsA) was also assessed. PBA showed tumor initiating activity while CBA demonstrated tumor initiating as well as promoting activities in two-stage mouse skin tumor protocol. Further, prior treatment of AsA to PBA and CBA followed by twice weekly application of 12-o-tetradecanoyl phorbal myristate acetate (TPA) resulted into delayed onset of tumor formation and similarly single application of 7,12-dimethylbenz [α] anthracene (DMBA) followed by twice weekly application of AsA and CBA showed an increase in the latency period. Thus, AsA showed a protective effect against CBA promoted skin tumor. Furthermore, the topical application of CBA significantly increased the levels of xenobiotic enzymes. The animals topically treated with AsA along with topical application of CBA, restored all the impairment observed in enzyme activities. Thus, this study suggested that AsA can be useful in preventing PBA and CBA induced skin tumorigenicity.
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1. Introduction

Benzanthrone (BA) presents a health threat to workers in the dye manufacturing units during the synthesis of number of vats and disperse dyes (NIOSH, 1979; Singh et al., 1990). BA has been detected in urban ambient air particulates originating from wood and coal combination gases, municipal refuse and auto-exhaust that may cause severe health complications (Randahl, 1983; Handa et al., 1984). BA-derived dyes/dye intermediates have been reported to cause dermal toxicity that appears to be influenced by the numbers of carbonyl and amino-anthraquinone groups as well as by the presence of functional groups like halogen, nitro, hydroxy and methoxy in the parent molecule (Singh et al., 2000). On the exposure to light, BA can generate active oxygen species that may be responsible for the photo contact dermatitis in the exposed industrial workers (Dabestani et al., 1992). In a skin-photosensitization study on guinea-pigs, BA has been reported to induce erythema and oedema in a dose dependent pattern (Srivastava et al., 1990). Dyestuff workers coming in contact with BA have been found to develop skin lesions, gastritis, liver malfunctions and sexual disturbances (Joshi et al., 1986; Schlede et al., 2003). Considering the aforementioned health complications induced by BA, it is quite likely that its long term exposure may lead to skin tumorigenicity. But, there is lack of a detailed study about the tumor initiating and promoting potentials of BA.

Dietary traditions and habits play important role in the causation and development of several human cancers (zur Hausen, 2012). However, a considerable number of dietary constituents have been found to protect against the occurrence of several neoplasia (Knasmüller et al., 2008). Naturally occurring dietary constituents like phenols, indoles, aromatic isothiocyanates, flavones, coumarines, sterols, tocopherols, retinol, carotenes and ascorbic acid (AsA) or vitamin C have shown inhibitory effects on the tumorigenesis (Khan et al., 2010). AsA is a water soluble vitamin that usually found in deprotonated state under the most physiological conditions. Being the most important antioxidant in extracellular fluids AsA participates in the several antioxidative evidents including lipid peroxidation and oxidative modification of low density lipoproteins (Steinbrecher et al., 1990). It has been reported that AsA can be used to accelerate the wound healing process (Hellman and Burns, 1958). AsA, the popular antioxidant available in the fruits and vegetables, has demonstrated its therapeutic efficacy in cancer therapy (Ullah et al., 2012). AsA can be used in the chemotherapy for reducing the toxic side effects without interfering the anticancer potential of the drugs (Fuchs-Tarlovsky, 2013; Park et al., 2012). Further, high doses (>10 g/day) of AsA have been used in the cold infections and in the cancer treatment (Cameron and Pauling, 1979; Naidu, 2003). In earlier reports, BA administration has been reported to cause significant depletion of AsA levels in the blood, adrenals and liver of rats, mice and guinea pigs (Pandya

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http://dx.doi.org/10.1016/j.fct.2013.07.014

et al., 1970). Therefore, it will be an interesting study to explore the therapeutic role of AsA in the BA exposed animals.

Since, BA exposed workers have been shown to exhibit many skin toxic manifestations, it was pertinent to examine the tumorigenic potentials (tumor initiating and promoting) of commercial benzanthrone (CBA) and purified benzanthrone (PBA) using a two stage skin tumor protocol and study the effects on some of the toxicity related marker enzymes. This study was further extended to explore the therapeutic role of AsA, on the tumorigenic potential and skin allergic markers induced by PBA and CBA.

2. Materials and methods

2.1. Animal

The Swiss Albino female mice (25 ± 5 g) obtained from CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow animal breeding colony, were raised on commercial pellet diet (Provimi Animal Nutrition India Pvt Limited, India) and water *ad libitum*. Animal study was carried out after the approval of animal ethics committee of CSIR-IITR, Lucknow, India.

2.2. Commercial and pure benzanthrone

Commercially available benzanthrone (CBA) is procured from Indian Dyestuff Company, Kalyan. CBA was further, purified on a neutral alumina column using 1,2-dichloroethane as eluant and the resulted product was termed as pure benzanthrone (PBA).

2.3. Animal treatment

The dorsal surface of mice were shaved with electric clipper (A5-00, Oster Corporation, Wisconsin, USA) and hair depilatory cream (Soft and Silky, Lakme, India) was applied 1 day prior to the beginning of the experiment. Mice were divided into 12 groups of fifteen each (15 mice/Group) and another 15 animals were taken in control group (Table 1). Skin tumor formation and body weight were recorded twice weekly for 30 weeks and tumors greater than 1 mm in diameter were included in the cumulative total, only if they persisted for 2 weeks or more. The latent periods were computed by the earlier described method (Andervont and Shimkin, 1940). In this study, we have used 7,12-dimethylbenz [α] anthracene (DMBA) and 12-o-tetradecanoyl phorbal myristate acetate (TPA) as a positive control for the tumor initiation and promotion, respectively.

2.4. Autopsy and histopathological processing

After the termination of experiments, the skin of mice form different grroups were immediately removed, washed with cold normal saline, soak dried over filter paper and weighed. A portion of the skin was fixed in 10% buffered formalin and embedded in paraffin after processing. Sections of 5 μm thicknesses were cut and stained with haematoxylin and eosin for further microscopic examinations.

2.5. Homogenization and enzyme preparation

Another portion of the skin samples were washed with cold 1.5% KCI, soak dried over filter paper. The skin fat from dermal side was removed, weighed and 10% homogenate was prepared in chilled 1.15% KCI solution using an Ultra Turrax polytron. The homogenates were filtered through a fine muslin cloth and either used as such or the supernatant (S-9) obtained on centrifugation of the homogenate at 10,000*g* for 20 min at 4 °C was used as enzyme source.

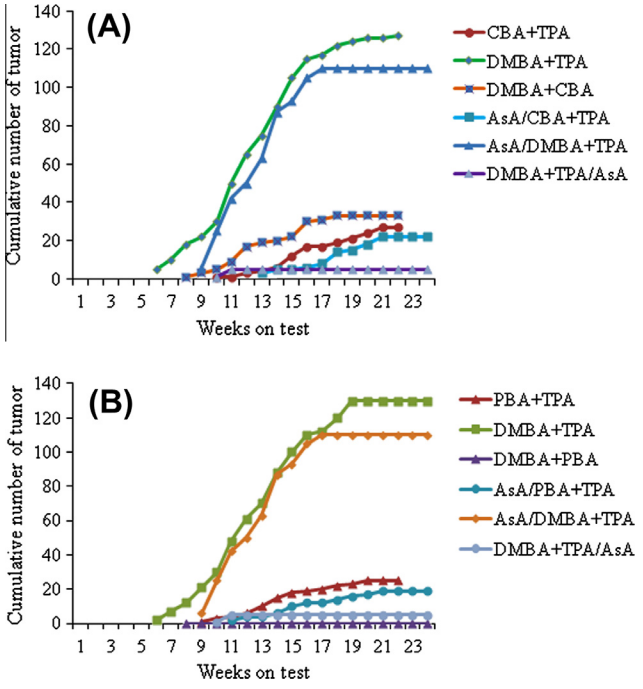

Fig. 1. Tumorigenic potential of CBA, PBA and its protection by AsA. (A) Tumor initiating and promoting activities of CBA in a two stage skin tumor protocol in mice and effect of AsA treatment prior to initiation, and promotion on skin tumor genesis. (B) Tumor initiating and proting activities of PBA in a two stage skin tumor protocol in mice and effect of AsA treatment prior to initiation, and promotion on skin tumor genesis. Where, PBA = pure benzanthrone; CBA = commercial benzanthrone; AsA = ascorbic acid.

2.6. Assays for aryl hydrocarbon hydroxylase (AHH), ethoxyresorufin-o-deethylase (ERD), glutathione-S-transferase (GST) and quinone reductase (QR)

The AHH, ERD, GST and QR were assayed according to the earlier described methods (Pohl and Fouts, 1980; Habig et al., 1974; Ernster, 1967), respectively.

2.7. Histidase and histidine decarboxylase levels

Histidase (histamine methyl transferase) and histidine decarboxylase or HOC (L-Histidine: 2-oxoglutarate amino-transferase) activities were assayed according to the earlier described methods (Tabor and Mehler 1955; Aures and Hakanson, 1970).

Table 1
Animal treatment protocols.

RZGroup	Dosing
Experiment 1: Tumor initiating potential of PBA and CBA and its protection by AsA	
Group-1	Vehicle control
Group-2	Single dose of DMBA (120 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Group-3	Single dose of PBA (750 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Group-4	Single dose of CBA (750 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Group-5	AsA (1 mg) application for 7 days followed by single dose of DMBA (120 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Group-6	AsA (1 mg) application for 7 days followed by single dose of PBA (750 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Group-7	AsA (1 mg) application for 7 days followed by single dose of CBA (750 nmol) + followed by twice weekly application of TPA (4 nmol) as promoter
Experiment 2: Tumor promoting potential of PBA and CBA and its protection by AsA	
Group-1	Single dose of DMBA (120 nmol) followed by twice weekly application of PBA (150 nmol) as promotor after a week of initiation.
Group-2	Single dose of DMBA (120 nmol) followed by twice weekly application of CBA (150 nmol) as promotor after a week of initiation
Group-3	Single dose of DMBA (120 nmol) followed by twice weekly application of AsA (1 mg) and TPA (4 nmol) after a week of initiation.
Group-4	Single dose of DMBA (120 nmol) followed by twice weekly application of AsA (1 mg) and PBA (150 nmol) was applied twice weekly as promotor after a week of initiation.
Group-5	Single dose of DMBA (120 nmol) followed by twice weekly application of AsA (1 mg) and CBA (150 nmol) after a week of initiation

where: PBA = pure benzanthrone; CBA = commercial benzanthrone; DMBA = 7,12-dimethylbenz [α] anthracene; AsA = ascorbic acid; TPA = 12-o-tetradecanoyl phorbal myristate acetate.

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