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Original research article

Chemopreventive effect of chrysin, a dietary flavone against benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice

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ABSTRACT

Background: Chemoprevention is considered as one of the most promising and realistic approaches in the prevention of lung cancer. Chrysin, a naturally occurring dietary flavone widely found in *Passiflora* family of plants and honey, has been studied extensively for its chemopreventive properties. The objective of present study is to divulge the chemopreventive role of chrysin against benzo(a)pyrene [B(a)P] induced lung carcinogenesis in Swiss albino mice.

Methods: B(a)P was administered orally (50 mg/kg body weight) twice a week for four weeks to induce lung cancer in mice. The body weight, lung weight, tumor incidence, lipid peroxidation, carcinoembryonic antigen, enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and non-enzymatic antioxidants (reduced glutathione, vitamin E and vitamin C) were estimated. Further, histopathological analysis of lung tissue and western blotting analysis of PCNA, COX-2 and NF-κB were also carried out.

Results: Administration of B(a)P resulted in increased lipid peroxides and carcinoembryonic antigen with concomitant decrease in the levels of both enzymatic antioxidants and non-enzymatic antioxidants. Chrysin treatment (250 mg/kg body weight) significantly attenuated all these changes thereby showing potent anti lung cancer effect. Further, the anticancer effect of chrysin was confirmed by histopathology of lungs, and immunoblotting analysis of PCNA, COX-2 and NF-κB, where chrysin supplementation downregulated the expression of these proteins and maintained cellular homeostasis.

Conclusion: Overall, these findings confirm the chemopreventive potential of chrysin against B(a)P induced lung cancer in Swiss albino mice.

Introduction

Lung cancer is the most prevalent cancer as well as leading cause of death in the world that represents a major public health problem [1]. As per the estimates given by Globocan 2012, it causes 1.8 million deaths in both men and women accounting for 20% of all cancer related deaths [2]. Benzo(a)pyrene (B(a)P), a prototype poly aromatic hydrocarbon, plays a major role in the etiology of lung carcinogenesis [3]. B(a)P is metabolized by cytochrome P450 (CYP) 1A1 to B[a]P-7,8-diol-9,10-epoxides (BPDE), the ultimate carcinogen. BPDE isomers covalently bind to the DNA; results in the formation of highly mutagenic DNA adducts (BPDE-N2-dG lesion) [4,5]. If these DNA adducts are not efficiently removed by detoxification processes and/or repaired by DNA repairing enzymes, this results in genetic alterations that ultimately contribute to the process of carcinogenesis. Additionally, B(a)P also metabolized to o-quinones by dihydrodiol dehydrogenases; which undergoes redox cycling with their semiquinone free radicals to form large amounts of reactive oxygen species (ROS), resulting in oxidative DNA damage, which plays a critical role in pathogenesis of B(a)P induced lung carcinogenesis [4,6].

Chemoprevention is regarded as one of the best ways of inhibiting, reversing or delaying carcinogenesis by using natural, dietary or synthetic agents [7]. A number of effective chemopreventive measures have been introduced considerably to decrease both the incidence and mortality owing to lung cancer;

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among them use of dietary agents has shown to be a promising approach [4]. A large number of epidemiological and experimental studies have suggested that diet plays a beneficial role in the chemoprevention of lung cancer [8–10]. Among dietary factors, certain flavonoid phytochemicals particularly those in the diet have marked lung cancer chemopreventive properties [11,12]. Flavonoids, the naturally occurring polyphenolic compounds consumed in human diet such as vegetables, fruits, nuts and beverages like tea, coffee and red wine [13], have demonstrated their chemoprevention and chemotherapeutic properties in many cancers.

Chrysin (5,7-dihydroxyflavone) (Fig. 1) is one such dietary phytochemical belongs to the class of flavonoids called flavones found in many plant extracts, including blue passion flower (*Passiflora caerulea*), honey and propolis, have great economic value and medicinal impact. It exerts a wide variety of pharmacological activities, including antioxidant [14], anti-inflammatory [15], anti-diabetic [16] and anticancer [17]. Laboratory studies also reveal that chrysin supplementation can substantially reduce cancer risk in animals treated with various chemical carcinogens [18]. These studies demonstrated that chrysin has promising potential as a chemopreventive agent and is worthy of further study. The purpose of our present investigation is to study the chemopreventive effect of chrysin on lung cancer induced by B(a)P in Swiss albino mice and to explore the possible mechanisms.

Materials and methods

Chemicals

B(a)P, chrysin and β-actin antibody were purchased from Sigma–Aldrich (USA). The primary and secondary antibodies used in this study were procured from Santa Cruz (USA). All other chemicals and reagents used were of the highest analytical grade procured from commercial sources.

Animals

Healthy male Swiss albino mice (18–22 g) of 6–8 weeks old were used throughout the study. They were housed in polypropylene cages and maintained in controlled environment conditions of temperature (24 ± 1 °C) and humidity (55 ± 5%) on alternatively 12 h light/dark cycles. They were fed standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai under the trade name Amrut rat/mice feed) and were given free access to water *ad libitum*. All the procedures with animals were strictly conducted according to the ethical norms approved by the Institutional Animal Ethical Committee regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental protocol

The experimental animals were divided into five groups, each group consisting of 6 animals as follows;

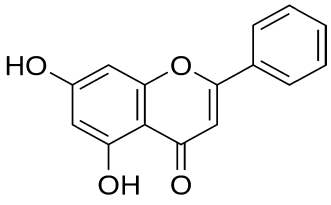

Fig. 1. Chemical structure of chrysin (5,7-dihydroxy-2-phenyl-4H-chromen-4-one) (CAS number: 480–40-0; molecular formula: $C_{15}H_{10}O_4$; molecular weight: 254.24 g/mol).

Group I (vehicle control) – received corn oil throughout the course of experiment.
Group II (chrysin drug control) – received chrysin (250 mg/kg body weight dissolved in corn oil) orally thrice in a week for 16 weeks to assess the cytotoxicity (if any) induced by chrysin.
Group III (B(a)P control) – received B(a)P (50 mg/kg body weight dissolved in corn oil) orally twice a week for four successive weeks [19].
Group IV (chrysin pre-treatment) – received B(a)P (as in Group III) along with chrysin (250 mg/kg body weight) orally thrice in a week for 16 weeks. Chrysin treatment was started one week prior to the first dose of B(a)P induction and continued till the end of experiment i.e., 16th week.
Group V (chrysin post-treatment) – received B(a)P (as in Group III) along with chrysin (250 mg/kg body weight) orally thrice in a week from 8th week after B(a)P induction and continued till the end of experiment i.e., 16th week.

Dose and dosing regimen for the present study was fixed based on previous studies [18]. The pre- and post-treated groups were used to study the chemopreventive as well as chemotherapeutic effect of chrysin (Fig. 2).

All the animals were weighed weekly once until the end of experimental period. After completion of the experimental period, animals from each group were anaesthetized and sacrificed by cervical decapitation. Immediately after euthanizing, blood was collected and serum was separated by centrifugation at 2500 × *g* for 10 min. The lungs were immediately dissected out, washed in ice-cold saline to remove any extraneous matter, cleaned and blotted to dry in filter paper. The weight of lungs and percentage of tumor incidence were observed. A 10% homogenate of lung tissue was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 12,000 × *g* for 30 min in a refrigerated high-speed centrifuge at 4 °C and the supernatant collected was stored at −80 °C until analysis. Total protein was estimated by the method of Bradford [20]. The following biochemical estimations were carried out in the lung supernatant and in the serum.

Lipid peroxidation and antioxidants

The levels of lipid peroxidation (LPO) were measured in serum and lung homogenates using method of Ohkawa [21]. From the lung tissue homogenates, the levels of enzymatic antioxidants such as superoxide dismutase (SOD) and catalase (CAT) were assayed using commercially available kits from Sigma (St Louis, MO, USA) according to the manufacturer specifications. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. [22]. Glutathione reductase (GR) was determined by the procedure described by Carlberg et al. [23]. Non-enzymatic anti-oxidants such as GSH [24], vitamin E [25] and vitamin C [26] were also measured.

Enzyme linked immunosorbent assay of carcinoembryonic antigen

Tumor marker enzyme carcinoembryonic antigen (CEA) was quantified by ELISA using the UBI MAGIWELL (USA) enzyme immunoassay kit according to the manufacturer specifications.

Lung histopathology

Histopathological assessment was performed on the lung tissues and a portion of specimen from all experimental groups was fixed in 10% buffered neutral formalin for one week at room temperature. Then tissues were dehydrated in a graded series of alcohol, cleaned in xylene and then embedded in paraffin. Tissue