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Naringenin ameliorates inflammation and cell proliferation in benzo(a)pyrene induced pulmonary carcinogenesis by modulating CYP1A1, NFκB and PCNA expression

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ABSTRACT

Lung cancer is the major cause of cancer-related mortality and is a growing economic burden worldwide. Chemoprevention has emerged as a very effective preventive measure against carcinogenesis and several bioactive compounds in diet have shown their cancer curative potential on lung cancer. Naringenin (NRG), a predominant flavanone found in citrus fruits has been reported to possess anti-oxidative, anti-inflammatory and anti-proliferative activity in a wide variety of cancer. The aim of the present study is to divulge the chemopreventive nature of NRG against benzo(a)pyrene (B[a]P) induced lung carcinogenesis in Swiss albino mice. Administration of B[a]P (50 mg/kg, p.o.) to mice resulted in increased lipid peroxidation (LPO), proinflammatory cytokines (TNF-α, IL-6 and IL-1β) with subsequent decrease in activities of tissue enzymic antioxidants (SOD, CAT, GPx, GR, GST) and non-enzymic antioxidants (GSH and Vit-C). Treatment with NRG (50 mg/kg body weight) significantly counteracted all these alterations thereby showing potent anti-cancer effect in lung cancer. Moreover, assessment of protein expression by immunoblotting and mRNA expression by RT-PCR revealed that NRG treatment effectively negates B[a]P-induced upregulated expression of CYP1A1, PCNA and NF-κB. Further, the antiproliferative effect of NRG was confirmed by histopathological analysis and PCNA immunostaining in B[a]P induced mice which showed increased PCNA expression that was restored upon NRG administration. Overall, these findings substantiate the chemopreventive potential of NRG against chemically induced lung cancer in mice.

1. Introduction

Lung cancer remains the most common cancer worldwide both in terms of incidence and mortality because of the high case fatality [1]. Globally, lung cancer is the largest contributor to new cancer diagnoses (1.82 million cases, 12.9% of total) and to death from cancer (1.6 million deaths, 19.4%) [1]. Even though the lung cancer incidence began declining in more developed regions as a result of reduced smoking, its incidence predominates in less developed regions where 58% (8 million) of the new cancer cases and 65% (5.3 million) of the total cancer deaths were reported as per the estimates of GLOBOCAN 2012 [1]. The 5-year total survival rate for lung cancer in the United States from 2004 to 2010 was 16.8%. Patients with localized disease at diagnosis have a 5-year survival rate of 54%; however, more than 57% of patients with distant metastasis at diagnosis have a dismal 5-year survival rate of 4% [2]. Although there has been some improvement in survival during the past few decades, the survival advances that have been realized in other common malignancies have yet to be achieved in lung cancer. Tobacco use is the principal risk factor for lung cancer, and a large proportion of all pulmonary carcinomas are attributable to the effects of cigarette smoking. The seminal report from the US Public health service estimated that the average male smoker had an approximately 9 to 10-fold risk for lung cancer, whereas heavy smokers had at least a 20-fold risk [3]. Cigarette smoke contains many potential carcinogens, including polycyclic aromatic hydro-carbons (PAHs), aromatic amines, N-nitrosamines, and other organic and inorganic compounds, such as benzene, vinyl chloride, arsenic, and chromium [3]. The prototype PAH, B[a]P is a significant pro-carcinogenic substance, which undergoes sequential metabolic activation principally by cytochrome P450 (CYP) 1A1 to generate a highly reactive carcinogenic metabolite B[a]P-7,8-diol-9,10-epoxides (BPDE) [4]. BPDE is capable of forming DNA adducts as well as chromosomal aberrations by binding to the guanine residues in DNA. Failure of the normal DNA repair mechanisms to remove these DNA adducts can lead to permanent mutations, DNA strand breaks, or other genetic alterations which contribute to the process of carcinogenesis [5].

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Chemoprevention has been advocated as an approach to reduce lung cancers with the idea of treating in the early steps of carcinogenesis. Chemoprevention is defined as the use of either natural or synthetic agents or their combination to interfere with the development of cancer cells by preventing the DNA damage that initiates carcinogenesis or by halting the progression of premalignant cells [6]. Flavonoids, the naturally occurring dietary antioxidants have engrossed a great deal of attention in recent years for their key role in the prevention of certain chronic diseases. Flavonoids have diverse biological activities including preventing the initiation through modulation of xenobiotic metabolism and halting the progression of carcinogenesis as a result of their anti-inflammatory, antioxidant, and anticancer properties [7]. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy [4]. Naringenin (4′, 5, 7-trihydroxy flavanone; NRG (Fig. 1)), a predominant flavanone found in grape and citrus fruits has a wide spectrum of pharmacological activities, including antioxidant, free radical scavenging, anti-inflammatory, immunomodulatory, anti-mutagenic and anti-carcinogenic effects [8, 9]. Previous reports demonstrate that NRG inhibits NDEA induced hepatocarcinogenesis through the downregulation of NF-κB, VEGF and MMPs, and also induce apoptosis by modulating expression of Bax, Bcl2 and Caspase-3 [10]. NRG has also shown protection against N-methyl-N′-nitro-N-nitrosoguanidine-induced gastric carcinoma by up-regulating the antioxidant defense enzymes [11,12]. Moreover, NRG acts as chemopreventive agent against colon carcinogenesis in vivo [13]. However, the effect of NRG on lung carcinogenesis is not proven till date. Hence, in the present study we investigated the chemopreventive and therapeutic efficacy of NRG against B[a]P induced lung carcinogenesis in Swiss albino mice.

2. Materials and methods

2.1. Drugs and chemicals

Naringenin, B[a]P, 2-thiobarbituric acid (TBA), trichloroacetic acid, reduced glutathione (GSH), 2,4-dinitrophenylhydrazine (DNPH), 5, 5′-dithiobis-2-nitrobenzoic acid, 1-chloro-2,4-dinitrobenzene (CDNB) and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Polyvinylidene difluoride (PVDF) membrane and 1-Step Ultra TMB blotting solution were purchased from Pierce Biotechnology, USA. Primary NF-κB (rabbit polyclonal) and PCNA (rabbit polyclonal) antibodies were purchased from Santa Cruz, USA. Primary anti-β-actin (rabbit monoclonal) and CYP1A1 (rabbit polyclonal) antibodies were procured from M/s Sigma Chemical Company, USA. The HRP-goat antirabbit IgG secondary antibody was obtained from Santa Cruz, USA. All other chemicals and reagents used were of analytical grade procured from Himedia Pvt. Ltd. (Mumbai, India).

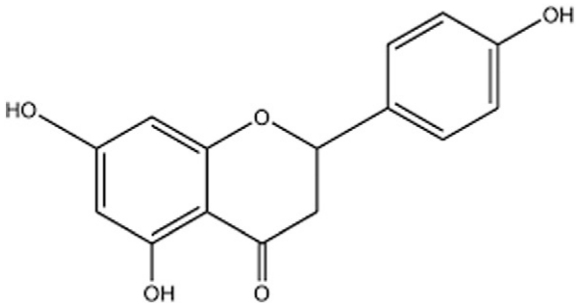

Fig. 1. Chemical structure of naringenin.

2.2. Animal model

Adult male Swiss albino mice weighing between 20 and 25 g (6–8 weeks old) were obtained from the central animal house facility of the institute. The animals were housed in plastic cages and maintained under standard conditions of temperature (25 ± 3 °C) and humidity (50 ± 10%) with a 12 h light–dark cycle. The animals had free access to a standard pellet diet and water ad libitum. All the procedures with animals were strictly conducted in accordance with approved guidelines by the Institutional Animal Ethical Committee regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3. Experimental design

Experimental animals were randomly divided into five groups, with six mice in each group. Group I (Normal control): Mice received corn oil throughout the course of the experiment. Group II (Drug control): Mice received NRG (50 mg/kg b.wt. dissolved in corn oil) orally, thrice in a week for 16 weeks. Group III (B[a]P control): Mice treated with B[a]P (50 mg/kg b.wt. dissolved in corn oil) orally twice a week for four successive weeks. Group IV (Pre-treatment): Mice received B[a]P (as in Group III) along with NRG (as in Group II) orally. NRG treatment was started one week prior to the first dose of B[a]P administration and continued for 16 weeks. Group V (Post-treatment): Mice received B[a]P (as in Group III) but NRG treatment was started from 8th week till the end of the experiment. The dose and dosing regimens of benzo(a)pyrene and hesperetin were fixed based on previous published literature [14–17].

Mice from each group were euthanized after 16 weeks by cervical decapitation under ether anesthesia. After euthanizing, lungs were immediately excised and washed with ice-cold saline. A 10% homogenate of the washed tissue was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 12,000 × *g* for 15 min in a refrigerated high-speed centrifuge at 4 °C and the supernatant collected was stored at −80 °C until analysis.

2.4. Biochemical estimations

The following biochemical estimations were carried out in lung homogenate. Lipid peroxides were estimated by the method of Ohkawa et al. in which the malondialdehyde (MDA) released served as the index of LPO [18]. GSH was assayed by the method as described by Ellman [19]. Superoxide dismutase (SOD) activity was determined using SOD assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer specifications. Catalase (CAT) activity was assayed by the method of Sinha [20], Glutathione peroxidase (GPx) was determined by the method of Rotruck et al. [21]. Glutathione reductase (GR) was assayed by the method of Carlberg and Mannervik [22]. Glutathione-S-transferase (GST) was assayed by the method of Habig et al. [23]. Vitamin C (Vit C) was measured by the method of Omaye et al. [24]. Total protein was estimated by the method of Bradford [25].

2.5. Estimation of pro-inflammatory cytokines

For estimation of proinflammatory cytokines (TNF-α, IL-6 and IL-1β) in lung tissues, a 10% tissue homogenate was prepared with phosphate buffer saline (0.01 M, pH 7.4) containing 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Then, the homogenates were centrifuged at 10,000 × *g* for 20 min and the supernatant obtained was used for the estimation of TNF-α, IL-6 and IL-1β using mouse TNF-α, IL-6 and IL-1β ELISA kits (Pierce Biotechnology, Rockford, IL, USA). The concentration of cytokines in lung tissue was expressed as ρg/mg protein.