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The Role of Antioxidant Anthocyanin in the Attenuation of Lung Cancer Caused by Benzo [A] Pyrene in Balb/C Mice

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Abstract: It was postulated that chemoprevention is one of the most promising and realistic approaches in the prevention of cancer. Anthocyanin (ANT) is one such naturally occurring flavonoid widely found in citrus fruits. The aim of the present study is to divulge the chemopreventive nature of ANT during Benzo[a]pyrene (B[a]p) induced lung cancer in BALB/c mice. Administration of B[a]p (60 mg/kg body weight) to mice resulted in increased lipid peroxidation (LPO), lung specific tumor marker carcinoembryonic antigen (CEA) and serum marker enzymes aryl hydrocarbon hydroxylase (AHH), alanine transaminase (ALT), with concomitant decrease in the levels of tissue antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione. ANT supplementation (25 mg/kg body weight) significantly attenuated these alterations thereby showing potent anticancer effect in lung cancer. Overall, these findings substantiate the chemoprotective potential of ANT against chemically-induced lung cancer in Balb/c mice.

Key words: Anthocyanin · Antioxidant · Benzo[A]Pyrine · Flavonoids · Lung Cancer

INTRODUCTION

B[a]p, a potential mutagen and carcinogen [1-4], is a member of the polycyclic aromatic hydrocarbon (PAH) family [5,6]. These compounds are formed from natural and man-made sources [7].

Lung cancer is the most prevalent cancer in the world that represents a major public health problem worldwide [8]. Tobacco smoking is well established as the major etiological risk factor for lung cancer, contributing to a tenfold increase in risk in long-term smokers compared with non-smokers [9]. Constituents of smoke, the polycyclic aromatic hydrocarbons (PAHs) such as B[a]p, play a major role in lung carcinogenesis [10]. B[a]p is metabolized to (±)-B[a]p-r-7,t-8-dihydrodiol-t-9,10-epoxide(BPDE), the ultimate carcinogen [11]. BPDE isomers then bind to the exocyclic nitrogen of deoxyguanosine in DNA via transaddition of the C-10 position in the epoxide molecule. This adduct might also cause activation of protooncogenes [12,13].

A number of effective chemoprevention measures have been introduced substantially to reduce both the incidence and mortality due to lung cancer.

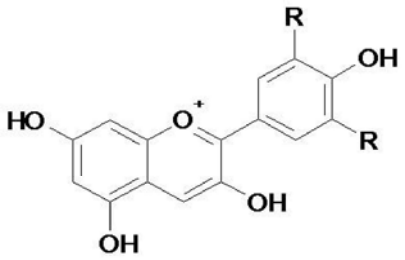

Fig. 1:

Recently, epidemiological studies have strongly suggested that consumption of antioxidants may reduce the risk of chronic diseases related to oxidative stress because of their antioxidant activity and promote general health benefits [14].

ANT (water soluble vacuolar pigment) (Fig. 1) belongs to the class of flavonoids called flavanones and is found in some fruits. It has several biological functions such as antioxidant, anti-inflammatory, prostaglandin-synthesis inhibition, anti-mutagenic activity and modulation of drug-metabolizing enzymes [15-18]. The effects of ANT in the prevention and treatment of diseases have recently received considerable attention

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[19]. Hence, the present study was aimed to elucidate the protective role of HDN on B[a]p induced lung cancer using different biomarkers in Balb/c mice.

MATERIALS AND METHODS

Chemicals: All chemicals used in this study were purchased from Sigma Co/ USA. Chemicals were of analytical grade.

Animals and Treatment: 40 Balb/c male mice (6-7 weeks old and around 30g weight each) were used in this study. Mice were kept on standard laboratory diet and tab water *ad libitum* throughout the experiments. Ten animals were housed stainless metal cages under 12:12h light-dark cycle and room temperature of 23-26°C. Four groups of mice (10 mice each) were assigned as: control group (CON), mice were received the drug vehicle, olive oil (2 ml/kg/day, orally) for 10 consecutive days. Group 2 animals were administered with B[a]p (50 mg/kg body weight dissolved in olive oil, orally) twice a week for 4 successive weeks to induce lung cancer by 16th week [20]. Third group of animals were treated with ANT alone (100 mg/kg body weight, orally) for 16 weeks on alternative days according to the method recommended in literature [21]. Group 4 animals were pretreated with ANT (as in group 3) one day before the first dose of B[a]p administration and continued for 16 weeks.

Biochemical Analysis: At the end of experimental period, mice were euthanized by cervical dislocation. The serum was separated from the collected blood. Lung tissues were immediately excised, weighed and then homogenized in 0.1 M Tris-HCl buffer (pH 7.4). Alanine transaminase (ALT) level was determined in the serum according to the method recommended before [22]. GSH content was evaluated using 5,5 -dithio-bis(2-nitrobenzoic acid) (Ellman's reagent, DTNB) [23]. Lipid peroxidation was assessed as malondialdehyde (MDA) content of lung tissue according to the method reported before [24], the marker enzyme aryl hydrocarbon hydroxylase (AHH) was estimated [25], gamma glutamyl transpeptidase (?-GT) [26]. Quantitative estimation of tumor marker, carcinoembryonic antigen (CEA) was based on solid phase enzyme linked immunosorbent assay (ELIZA) [27]. Toxicity of reactive oxygen species (ROS) was estimated as reported before [28]. Total phenolic component of ANT was determined according to the method recommended in Simon *et al.* [28], the DPPH radical scavenging activity of ANT was determined according to Hadizadeh *et al.* [29], the reducing power of the extract was quantified by the method described before [30] using BHT as standard antioxidant. Activities of antioxidant enzymes, SOD, CAT and glutathione peroxidase (GSHpx) were determined by the methods reported in literature [31].

Statistical Analysis: Results are expressed as mean ± standard deviation. For comparison between groups, data were analyzed by one-way ANOVA; P= 0.05 was considered statistically significant.

RESULTS

The present results revealed that the final body weight of the B[a]p administered animals was significantly ($P \leq 0.05$) lowered than that of control group, while the lung weight, tumor incidence was significantly increased ($P \leq 0.05$). Treatment with ANT increased ($P \leq 0.05$) the final body weight and significantly decreased lung weight and tumor incidence in group 4. B[a]p-treated mice liver showed a little increase in the liver body mass index ratio due to massive intra-hepatic hemorrhage and pooling of blood in the liver, making the liver appear darker in colour when compared with the other groups, which were all within the normal values as shown in Table 1.

Table 2 depicts the levels of the tumor marker CEA in control and experimental animals. CEA levels were found to be significantly increased (P= 0.05) in B[a]p-induced lung cancer bearing animals (B[a]p group) but were significantly lowered in HDN-treated group. The activities of marker enzymes, AHH, y-GT and ALT were found to be significantly (P=0.05) increased in lung cancer bearing

Table 1: Effects of ANT on the body weight, liver weight and lung weight of control and experimental animals

Particulars	Group 1 (CON)	Group 2 B[a]p	Group 3 (ANT)	Group 4 (ANT+b[a]p)
Body weight(g)	31.4±2.77	19.6±2.14	32.1±3.22	26.8±2.69
Liver weight (g)	1.55±0.03	2.33±0.06	1.49±0.02	1.71±0.06
Lung weight (mg)	267±22.23	324±27.88	255±25.62	289±26.44
Tumor incidence	0	7	0	2

Table 2: Effect of ANT on the levels of carcinoembryonic antigen (CEA) and on the activities of marker enzymes in the serum of control and experimental animals

Particulars	Group1 (CON)	Group 2 (B[a]p)	Group 3 (ANT)	Group 4 (ANT+B[a]p)
CEA (ng/ml)	0.6±0.02	5.8±0.08	0.3±0.01	1.8±0.03
AHH (μmol/min/mg protein)	0.3±0.01	1.2±0.02	0.02±0.01	0.6±0.01
□-GT (nmoles/min/mg protein)	1.1±0.01	2.8±0.06	0.8±0.01	1.6±0.02
ALT (U/L)	13.8±0.11	28.4±0.22	11.8±0.08	18.7±0.15

Table 3:Effect of ANT on the antioxidant enzymes, LPO and GSH level

Particular	Group 1(CON)	Group 2(B[a]p)	Group 3(ANT)	Group 4 (ANT+B[a]p)
LPO (nmol/mg of wet tissue)	0.77±0.01	6.67±0.12	0.71±0.01	2.08±0.03
SOD (U/mg)	47.61±2.87	28.33±1.21	48.05±2.67	44.21±2.46
CAT (U/mg)	224±31.32	122±17.27	231±36.87	191±32.08
Gpx (μmoles/min/mg)	48.66±4.62	17.75±2.64	51.10±4.44	37.81±2.77
GSH (μg/mg protein)	1.61±0.22	0.81±0.14	1.66±0.20	1.13±0.16

which were reversed to near normal in HDN treated animals (Group 4). However, no significant difference was observed between the HDN-treated group and control animals.

The levels of lipid peroxides, cellular enzymatic antioxidants such as SOD, CAT and GPx as well as GSH in lung tissues of the various experimental groups are presented in Table 3. A highly significant (P=0.05) increased in tissue LPO with concomitant decrease in the activity of enzymatic and non-enzymatic (GSH) antioxidants was observed in tumor bearing animals. These adverse changes were reversed to near normal values in ANT-treated animals.

DISCUSSION

B[a]p is a contaminant that occurs ubiquitously in the environment together with other PAHs as a product of incomplete combustion or pyrolysis of organic materials containing carbon and hydrogen. Main sources of B[a]p and PAHs in the environment are residential heating, industrial plants, vehicle exhausts and cigarette smoke [34]. It was found by other researchers [35, 32] that polycyclic aromatic hydrocarbons PAHs such as b[a]p, play a major role in lung carcinogenesis.

Enzymatic antioxidants such as SOD, CAT and GPx synergistically scavenge reactive oxygen species and prevent LPO. Several reports have cited decreased activities of SOD and CAT in various carcinogenic conditions [36]. GPx is a well-known first line of defense against oxidative stress, it catalyzes the transformation of H_2O_2 to harmless by products, thereby curtailing the quantity of cellular destruction and several studies have reported the decreased activities of GPx in various cancerous conditions [3]. Antioxidant enzymes constitute a mutually supportive team of defense against ROS. These enzymes have been found to be decreased in B[a]p induced lung cancer bearing experimental animals [37]. Neoplastic cells may sequester essential antioxidants from circulation to supply the demands of growing tumor [30, 36]. Antioxidant vitamins have a number of biological activities such as immune stimulation, scavenging the free radicals and alteration in metabolic activation of carcinogens [38]. They can utilize reactive oxygen metabolites, protecting biopolymers and reduce oxidative DNA damage [39]. The B[a]p is a very effective carcinogen with a capability to induce enormous amounts of free radicals and ROS [39].

To our knowledge the potential protective effect of ANT as antioxidant on lung cancer has not yet been evaluated. In this sense, we have addressed the possible protective effects of the citrus flavonoid ANT in such B[a]p-induced lung cancer. The B[a]p dose selected in the current work is well correlated with the environmental nutritional exposure limits in humans. Indeed, up to 85 mg of B[a]p total burden has been reported in various dietary intakes.

Analysis of serum marker enzymes serves as an indicator of cancer response to environment. Distribution of many biochemical, immunological and molecular properties of the host have been observed in B[a]p mediated cancer conditions [38]. Marker enzymes such as AHH, GGT and LDH are specific indicators of lung damage [23]. Chen and Liu [8] reported that AHH is one of the useful biomarkers in early diagnosis of lung cancer. GGT is not useful in diagnosis but also has extrapolative value in malignancies such as lung cancer and malignant melanoma. An increased level of GGT was observed in cancer cells [17]. This elevation may indicate the basic tumor burden. LDH is a fairly sensitive marker for solid

neoplasms and elevated activity of the enzyme was reported in serum of lung cancer patients [28]. The possible reason for elevated levels of LDH may be due to higher glycolysis in cancerous conditions.

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