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Original article

The potency of essential nutrient taurine on boosting the antioxidant status and chemopreventive effect against benzo (a)pyrene induced experimental lung cancer

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ABSTRACT

Lung cancer is a major cause of morbidity and mortality worldwide both in men and women accounting for 29% of all other cancers. The constituents of smoke consist of polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene which play a major role in lung carcinogenesis. B(a)P increases oncogenic stimulation by enhancing intrinsic ROS stress and metabolic activity. Recent focus of cancer chemoprevention is on the supplementation of natural anti-oxidants which are capable of ameliorating biochemical and molecular changes that occur during carcinogenesis. Taurine (2-aminoethanesulfonic acid), a sulfur-containing β-amino acid abundant in sea foods has potent antioxidant property. The present study was framed to investigate the potency of taurine on boosting the antioxidant status and chemopreventive effect against benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice. Administration of B(a)P (50 mg/kg body weight) to mice resulted in decrease in the activities of enzymic and non-enzymic antioxidants with concomitant increase in lipid peroxides (LPO), protein carbonyls and lung specific tumor markers. Taurine supplementation (100 mg/kg body weight) significantly attenuated these alterations. From these results, we suggest that administration of B(a)P induces ROS production and diminishes antioxidant levels. Conversely, taurine affords protection from ROS induced lung damage by augmenting the function of anti-oxidants.

1. Introduction

Lung cancer was a rare disease until the beginning of the 20th century. Since then, its incidence has increased rapidly and this neoplasm has become the most common cancer in men in most countries, and is the major cause of cancer death worldwide. Globally, it is estimated that there are approximately 13 million new cancer cases and 8 million cancer deaths. Worldwide, lung cancer is the most common cancer in terms of both incidence and mortality (1.61 million new cases per year and 1.38 million deaths). In India, as per the estimates, there are approximately 63,000 new lung cancer cases reported each year, though the incidence (ASR per 100,000) rates are low (M = 7.4, F = 1.8), compared to the rates in other parts of the world [1].

Tobacco contents of smoke, the polycyclic aromatic hydrocarbons (PAHs) such as B(a)P, that play a major role in induction of lung carcinogenesis [2]. B(a)P is metabolized to (±)-B(a)P-r-7,t-8-dihydrodiol-t-9,10-epoxide (BPDE), the ultimate carcinogen. BPDE isomers then bind to the hexocyclic nitrogen of deoxyguanosine in DNA via trans-addition of the C-10 position in the epoxide molecule. This adduct might also cause activation of proto-oncogenes [3].

A number of effective chemoprevention measures have been introduced substantially to reduce both the incidence and mortality due to lung cancer. The search for new compounds in foods or in plant medicines showing anticancer effects is one realistic and promising approach to prevention [4].

The intake of antioxidant compounds, or compounds that ameliorate or enhance the biological antioxidant mechanisms can prevent and in some cases help in the treatment of some oxidative-related disorders and organ toxicity events [5]. Taurine is the major intracellular free β-amino acid in most mammalian tissues and known as a conditionally essential nutrient, which can be synthesized from methionine and cysteine and obtained largely from the diet, predominantly through eggs, meat and seafood [6]. The beneficial effects of taurine as an antioxidant in biological systems have been attributed to its ability to stabilize biomembranes [7], scavenge reactive oxygen species [8], and reduce the production of lipid per oxidation end products [9].

Hence, the present study was designed to elucidate biochemical anomalies associated with lung cancer and also to validate

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the anticancer effect of taurine against B(a)P-induced lung carcinogenesis in Swiss albino mice.

2. Materials and methods

2.1. Animals

The experimental model is attractive as a screening tool because a substantial overlap exists between man and mouse in the genetic alterations thought to be responsible for lung tumorigenesis. Healthy male Swiss albino mice (7–8 weeks old) weighing 20–25 g were used throughout the study. Mice were acclimated to laboratory condition with regular temperature control ranging from $23 \pm 2\,^{\circ}C$ and were given ad libitum access to balanced diet (Gold Mohor rat feed, Ms. Hindustan Lever Ltd., Mumbai) and water. All the experiments were performed in compliance with the regulation of our institutional Animal Care and Use Committee. They were maintained in a controlled environment condition of alternative12 h light/dark cycles. This research work on male Swiss albino mice was sanctioned and approved by our Institutional animal ethical committee (IAEC/No-01/026/10).

2.2. Drugs and chemicals

Benzo(a)pyrene and Taurine were purchased from Sigma Chemical Company, USA. All other chemicals used were of analytical grade obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Glaxo Laboratories, CDH division, Mumbai, India.

2.3. Experimental protocol

The animals were divided into five groups consisting of eight animals in each group. Group I served as control animals and received corn oil as vehicle. Group II animals were treated with benzo(a)pyrene (50 mg/kg body weight, dissolved in corn oil orally twice a week for four consecutive weeks, from week 2 to 6). Group III animals were subjected to pre-treatment with taurine (100 mg/kg body weight, dissolved in corn oil, given orally) from the 1st to 18th week twice a week as benzo(a)pyrene. Benzo(a)pyrene was administered to the animals simultaneously from the week 2 to 6 for induction of lung cancer. Group IV animals were treated with taurine (100 mg/kg body weight, dissolved in corn oil) from week 12 of benzo(a)pyrene (as in group II) induction up to the end of the experimental period. Group V animals were treated with taurine alone (as above) for 18 weeks.

2.4. Anti-oxidants

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Lung tissues were immediately excised, weighed and then homogenized in 0.1 M Tris–HCl buffer (pH 7.4). Both homogenate and serum were taken for the analysis as described below. Total protein was estimated by the method of Lowry et al. [10]. The activity of superoxide dismutase (SOD) was estimated by the method of Marklund and Marklund [11]. The enzyme activity is defined as units/mg protein. The activity of catalase (CAT) was estimated by the method of Sinha et al. [12] and expressed as nmol of H_2O_2 consumed/min/mg protein. Activity of glutathione peroxidase (GPX) was assayed by the method of Rotruck et al. [13]. Enzyme activity was expressed as mmol of GSH oxidised/min/mg protein. The activity of glutathione reductase (GR) was assayed by the method of Stahl et al. [14] and expressed as nmol of NADPH oxidized/min/mg protein. Non-enzymic antioxidants, such as GSH was measured by the method of Moron et al. [15] Vit. E estimated by the method of Desai [16] and Vit. C measured by the method of Omaye et al. [17] was also measured and units were expressed as μg/mg protein.

2.5. LPO measurement

LPO was measured using the procedure of Uchiyama and Mihara [18]. The lung tissues were homogenized in chilled 0.1 mol/l potassium chloride solution. The mixture consisted of 10 mmol/l BHT, 0.67% TBA, 1% chilled OPA and tissue homogenate (10%). The mixture was incubated at $90\,^{\circ}C$ for 45 minutes. The absorbance of supernatant was measured at 535 nm. The rate of LPO was determined as nmol of TBA reactive substances (TBARS) formed/h/g of tissue using a molar extinction coefficient of 1.56×105 l/mol cm.

2.6. Determination of carbonyl content

Carbonyl content of proteins was determined by the DNPH method as described by Levine et al. [19] with some modifications. A 10% tissue homogenate is prepared in 5 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA. The homogenate was centrifuged at $15{,}000 \times g$ for 80 minutes at 4○ C. The proteins in the supernatant were precipitated with 10% trichloroacetic acid (TCA) followed by centrifugation at 5000 rpm for 5 minutes. The precipitates were incubated with 10 mM DNPH. The reaction mixture was allowed to stand for 1 h at room temperature with stirring at 15 minutes interval. After the incubation, the mixture was centrifuged at 5000 rpm for 5 minutes. The precipitates were washed with an ethanol–ethylacetate (1:1) mixture three times and the final precipitates were dissolved in 6 M guanidine hydrochloride. The absorbance was measured at 360 nm and the carbonyl content was obtained as nmole per mg protein using a molar extinction coefficient of 22×103.

2.7. Enzyme linked immunosorbent assay (ELISA) of CEA

Quantitative estimation of tumor marker, carcinoembryonic antigen (CEA) was based on solid phase enzyme linked immunosorbent assay (ELISA) using the UBI MAGIWELL (USA) enzyme immunoassay kit.

2.8. Statistical analysis

Data are presented as the mean ± standard deviation (SD). Oneway analysis of variance (ANOVA) was used to detect the significant changes between the groups. The student least significant difference (LSD) method was used to compare the means of different groups and the significance was denoted by '*P*' value.

3. Result

3.1. Body weight and lung weight

Table 1 shows the effect of taurine on the body weight and lung weight in control and experimental group of animals. Induction of lung cancer by B(a)P in mice (Group II) resulted in loss of body weight when compared to control mice. On the contrary, the lung weight was significantly increased ($P < 0.05$) than that of control group of (Group I) animals. Administration of taurine to B(a)P-induced mice (Groups III & IV) showed significant ($P < 0.05$) increase in the body weight and lowered lung weight when compared with B(a)P-induced mice (Group II). No obvious changes were observed between the control and taurine alone treated group which is indicative of nontoxic nature of taurine.