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ScienceDirect
Biomedicine & Pharmacotherapy 62 (2008) 390–394
BIOMEDICINE & PHARMACOTHERAPY
www.elsevier.com/locate/biopha

Original article

Stabilization of pulmonary mitochondrial enzyme system by capsaicin during benzo(a)pyrene induced experimental lung cancer

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Received 14 September 2007; accepted 25 September 2007
Available online 25 October 2007

Abstract

The modulatory efficacy of capsaicin on lung mitochondrial enzyme system with reference to mitochondrial lipid peroxidation (LPO), antioxidants, key citric acid cycle enzymes and respiratory chain enzymes during benzo(a)pyrene (B(a)P) induced lung cancer in Swiss albino mice was studied. Elevations in mitochondrial LPO along with decrements in enzymic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST)), non-enzymic antioxidants (reduced glutathione (GSH), vitamin C, vitamin E and vitamin A), citric acid cycle enzymes (isocitrate dehydrogenase (ICDH), alpha-ketoglutarate dehydrogenase (alpha-KDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH)), and respiratory chain enzymes (NADH dehydrogenase and Cytochrome *c* oxidase) were observed in B(a)P (50 mg/kg body weight) administered animals. CAP (10 mg/kg body weight) pretreatment decreased lung mitochondrial LPO and augmented the activities of enzymic, non-enzymic antioxidants, citric acid cycle enzymes and respiratory chain enzymes to near normalcy revealing its chemoprotective function during B(a)P induced lung cancer.

Keywords: Lung cancer; Capsaicin; Mitochondrial lipid peroxidation and antioxidants; Citric acid cycle enzymes; Respiratory chain enzymes

1. Introduction

The statistics on lung cancer imposes the urge to extend new methods to control this deadly form of cancer. In recent years phytochemicals have fascinated the attention of researchers because they show promise of being powerful antioxidants that can protect human beings from free radical induced toxic effects [1]. Epidemiological data have indicated favorable effects of antioxidant compounds in the prevention of a multitude of disease status, including cancer, cardiovascular diseases and neurodegenerative diseases [2]. These natural compounds cannot be produced by the human body and thus must be taken in, mainly through the diet.

Pepper fruits (*Capsicum annuum* L.) are important vegetables used as vegetable foods and as spices with well-known antioxidant properties [3]. Capsaicin (CAP) (trans-8-methyl-*N*-vannilyl-6-nonemide) is the major pungent principle found in hot red and chilli peppers of the plant genus *Capsicum* that has long been used as spices, food additives and drugs [4]. This alkaloid component CAP has recently reported to retain protective properties against experimental carcinogenesis and mutagenesis [5]. In addition, CAP has been reported to show antioxidant action in experimental conditions [6].

Free radical and non-radical oxidizing species are frequently produced in animals treated with carcinogens and mounting evidence suggests that these free radicals and electrophiles mediated oxidative stress plays an important role in all stages of chemical carcinogenesis and tumorigenesis [7]. Benzo(a)pyrene (B(a)P), a well-recognized lung carcinogen present in tobacco smoke, is known to induce enormous amounts of free radicals, which in turn reacts with lipids causing release of lipid peroxides and involvement of free radicals in B(a)P induced lung carcinogenesis was confirmed by the overproduction of 8-hydroxy guanine in lung and liver of B(a)P administrated mice [8].

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doi:10.1016/j.biopha.2007.09.005

Recent reports revealed that CAP inhibits potentially various lipid peroxidations, directly scavenges different toxic radicals and hampers the accumulation of reactive oxygen species and the radical chain reaction in a concentration dependent manner [9]. CAP pretreatment was also found to protect against the free radical induced pulmonary damage in rats exposed to gaseous chemical irritants such as sulfur dioxide and nitrogen dioxide [10].

Therefore, the primary object of the present study is to assess the beneficial role of CAP in modifying the pulmonary mitochondrial enzyme system with respect to LPO, antioxidant status, major citric acid cycle enzymes and respiratory chain enzymes during B(a)P induced lung carcinogenesis.

2. Materials and methods

2.1. Materials

Benzo(a)pyrene and capsaicin were purchased from Sigma chemicals, St. Louis, USA. All other chemicals were of analytical grade procured from local commercial sources.

2.2. Animal model

Healthy male Swiss albino mice weighing 20–25 g (8–10 weeks old) obtained from Veterinary College, Chennai, were used throughout the experiment. Animals were utilized as per the guidelines from the Institutional Animal Ethics Committee. The animals were housed under conditions of controlled temperature (26 ± 2 °C) with 12-h day/night cycle. They were fed standard rat/mice pellet diet (M/s. Hindustan Lever Ltd., Mumbai) under the trade name Amrut rat/mice feed and were given access to water *ad libitum*.

2.3. Experimental design

Experimental animals were divided into four groups of six mice each as follows: Group I (control) received olive oil throughout the course of the experiment; Group II (B(a)P) were treated with benzo(a)pyrene (50 mg/kg b.wt. dissolved in olive oil) orally twice a week for four successive weeks; Group III (CAP) received capsaicin (10 mg/kg b.wt. dissolved in olive oil) intraperitoneally once in a week for 14 weeks to assess the cytotoxicity (if any) induced by CAP and Group IV (B(a)P + CAP) received B(a)P (as in Group II) along with capsaicin (10 mg/kg b.wt. dissolved in olive oil) intraperitoneally. CAP treatment was started one week prior to the first dose of B(a)P administration and continued for 14 weeks. This dose of CAP is set based on effective dosage fixation studies.

At the end of the experimental period (14th week), animals were sacrificed by cervical decapitation under ether anesthesia and lungs were excised immediately and washed with ice-cold saline. A 10% homogenate of the washed tissue (lung) was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of $12{,}000 \times g$ for 30 min in a refrigerated high-speed centrifuge at 4 °C. The following biochemical estimations were carried out in the supernatant.

2.4. Biochemical analysis

Lung mitochondria were isolated by the method of Johnson and Lardy [11] and the following parameters were analysed. Protein was estimated by the method of Lowry et al. [12]. LPO was assayed by the method of Ohkawa et al. [13] in which the malondialdehyde (MDA) released served as the index of LPO. Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund [14]. Catalase (CAT) activity was assayed by the method of Sinha [15], glutathione peroxidase (GPx) was determined by the method of Rotruck et al. [16]. Glutathione reductase (GR) was assayed by the method of Beutler [17]. Glutathione-S-transferase (GST) was assayed by the method of Habig et al. [18]. Reduced glutathione (GSH) was assayed by the method of Moron et al. [19], vitamin E was estimated by the method of Desai [20], vitamin C was measured by the method of Omaye et al. [21] and vitamin A was determined by the method of Bayfield and Cole [22]. Citric acid cycle enzymes isocitrate dehydrogenase (ICDH) was assayed by the method of King [23], alpha-keto dehydrogenase (alpha-KDH) by the method of Reed and Mukherjee [24], succinate dehydrogenase (SDH) by the method of Slater and Bonner [25] and malate dehydrogenase (MDH) by the method of Mehler et al. [26]. The activities of respiratory chain enzyme NADH dehydrogenase was determined by the method of Minakami et al. [27] and Cytochrome *c* oxidase by the method of Pearl et al. [28].

2.5. Data analysis

All data were expressed as mean ± SD for six mice. The results were computed statistically (SPSS Software Package) using one-way ANOVA. Post hoc testing was performed for inter-comparisons using the LSD. $P < 0.05$ was considered significant.

3. Results

Table 1 depicts the effect of B(a)P and CAP on lung mitochondrial LPO and enzymic antioxidant status. Significant ($P < 0.05$) increase in LPO with concomitant decrease in the activities of enzymic antioxidants SOD, CAT, GPx, GR and GST was observed in the B(a)P administered group (Group II). CAP pretreatment resulted in a free radical quenching effect and thereby significantly ($P < 0.05$) decreasing LPO and reinstating the enzymic antioxidant activities to near normalcy in Group IV animals.

Table 2 shows the levels of non-enzymic antioxidants GSH, vitamin C, vitamin E and vitamin A in control and experimental group of animals. B(a)P treatment markedly ($P < 0.05$) reduced the levels of GSH, vitamin C, vitamin E and vitamin A in Group II animals that were significantly ($P < 0.05$) restored to near normalcy on CAP pretreatment in Group IV animals.

The influence of CAP on the activities of key citric acid cycle enzymes and respiratory chain enzymes is shown in Table 3. The activities of the citric acid cycle enzymes