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Beneficial influence of capsaicin on lipid peroxidation, membrane-bound enzymes and glycoprotein profile during experimental lung carcinogenesis

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Abstract

This study was designed to examine the impact of a principal component of hot red peppers and chilli peppers, capsaicin, on alterations in lipid peroxidation, membrane-bound enzyme profiles and glycoprotein levels during benzo(a)pyrene (BP)-induced lung cancer in Swiss albino mice. BP (50 mg kg^{-1}) induced deleterious changes that were revealed by alterations in lipid peroxidation, membrane-bound enzyme (Na^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase) activity, levels of total protein and protein-bound carbohydrate components (sialic acid, hexose, hexosamine, hexuronic acid and fucose). Pre-co-treatment with capsaicin (10 mg kg^{-1}) restored the detrimental effects induced by BP, indicating its protective role in BP-induced lung cancer.

Introduction

The severity and magnitude of the cancer problem makes it imperative to develop chemopreventive strategies utilizing pharmacological or natural agents to block the initiation or to arrest the progression of pre-malignant cells. Numerous studies have found that chemoprevention can avert a wide variety of cancers in many animal models (Tanaka 1994; Morse & Stoner 1993; Pezzuto 1997). Many phenolic substances in the human diet have been shown to exert substantial chemopreventive effects against experimental carcinogenesis and mutagenesis (Ferguson 1994). The identification of dietary or non-dietary natural products as cancer chemopreventive agents has been hailed by many investigators to be practically beneficial, especially when the carcinogenic insult is mild to moderate (Singh et al 2006).

Capsaicin (8-methyl-*N*-vannilyl-6-nonenamide) is the major pungent principle found in hot red peppers and chilli peppers of the plant genus Capsicum, and has long been used as a spice, food additive and drug (Cordell & Araujo 1993). This alkaloid compound has attracted considerable attention because of its chemoprotective properties against certain carcinogens and mutagens (Surh & Lee 1995). In addition, capsaicin has been reported to show antioxidant action in experimental conditions both in-vivo and in-vitro (Okada & Okajima 2001; Lugman & Rizvi 2006). This natural compound is also reported to have anti-inflammatory, antifungal and analgesic effects (Chowdary et al 1996), as well as proven effectiveness against gastric (López Carillo et al 1994), liver (Miller et al 1993) and duodenal cancers (Kang et al 1995). Capsaicin pretreatment was also found to protect against free radical induced pulmonary damage in rats exposed to gaseous chemical irritants such as sulfur dioxide and nitrogen dioxide (De & Ghosh 1993). Kogure et al (2002) reported that capsaicin potentially inhibits lipid peroxidation (LPO), directly scavenges different toxic radicals and reduces the accumulation of reactive oxygen species and the radical chain reaction in a concentration-dependent manner.

The present study aimed to determine if capsaicin pretreatment can alleviate the toxic effects produced during benzo(a)pyrene (BP)-induced lung cancer, using LPO, membrane-bound enzymes and protein-bound carbohydrates as biochemical endpoints of chemoprevention.

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Materials and methods

Materials

BP and capsaicin were purchased from Sigma Chemicals (St Louis, MO, USA). All other chemicals were of analytical grade and were procured from SRL Chemicals Pvt Ltd (Mumbai, India).

Animals

Healthy male Swiss albino mice, 20–25 g (8–10 weeks old), obtained from the Veterinary College, Chennai, India, were used in the experiment. The study was approved by the Ministry of Social Justices and Empowerment, Government of India and by the Animal Ethics Committee Guidelines of the University of Madras, India. The animals were housed under conditions of controlled temperature (26 ± 2°C), with a 12-h day–night cycle. They were fed a standard pellet diet (Amrut rat/mice feed; M/s. Hindustan Lever Ltd, Mumbai, India) and were given free access to water.

Experimental design

Experimental animals were divided into four groups of six mice each. Group 1 (control) received olive oil throughout the course of the experiment. Group 2 were treated with BP (50 mg kg^{-1} dissolved in olive oil) orally twice a week (Day 1 and Day 4) for four successive weeks. Group 3 received capsaicin (10 mg kg^{-1} dissolved in olive oil) intraperitoneally once a week for 14 weeks to assess the cytotoxicity, if any, induced by capsaicin. Group 4 (BP + capsaicin) received BP (as for Group 2) along with capsaicin (10 mg kg^{-1} dissolved in olive oil) intraperitoneally. Capsaicin treatment was started 1 week before the first dose of BP and continued for 14 weeks. The dose of capsaicin was chosen based on our previous study (Anandakumar et al 2008).

Biochemical analysis

At the end of the experimental period, the animals were killed by cervical decapitation. Blood and lung tissues were collected; tissues were immediately excised, weighed and then homogenized in 0.1 M Tris-HCl buffer (pH 7.4). Erythrocyte membranes were isolated according to the method of Dodge et al (1963). Analysis of total protein in serum and tissue homogenates was done according to Lowry et al (1951). LPO was assayed according to Ohkawa et al (1979), with malondialdehyde (MDA) release serving as the index of LPO.

Determination of membrane-bound enzymes

Na^+/K^+ATPase activity was determined according to Bonting (1970). The activity of Ca^{2+}ATPase (Hjerten & Pan 1983) and Mg^{2+}ATPase (Ohnishi et al 1982) was assayed. The inorganic phosphorus was estimated according to Fiske & Subbarow (1925).

Assay of glycoproteins

Hydrolysis of glycoproteins for the determination of hexose, hexosamine, hexuronic acid and fucose was carried out. A known amount of defatted tissue was put into a test tube to which 1 mL of 2 N HCl was added, and the tubes were sealed. Hydrolysis was completed by keeping the sealed tubes at 100°C for 16–18 h. After hydrolysis, the contents were neutralized with NaOH and made up to a known volume, and aliquots were used for hexose, hexosamine, hexuronic acid and fucose determination. Hexose, hexosamine, hexuronic acid and fucose were determined according to Niebes (1972).

Analysis of sialic acid

A known amount of defatted tissue was hydrolysed with 0.1 N H_2SO_4 at 90°C and neutralized. The hydrolysed extract was used for the determination of sialic acid (Warren 1957).

Statistical analysis

All data were expressed as mean ± s.d. for six mice. The results were analysed by one-way analysis of variance using SPSS software (Chicago, IL, USA). Post-hoc testing was performed for inter comparisons using the LSD test. $P < 0.05$ was considered significant.

Results

Figure 1 shows the effect of BP and capsaicin on erythrocyte membrane, lung and serum LPO in control and experimental animals. A significant increase in LPO measured in terms of malondialdehyde levels was observed in the BP-treated group

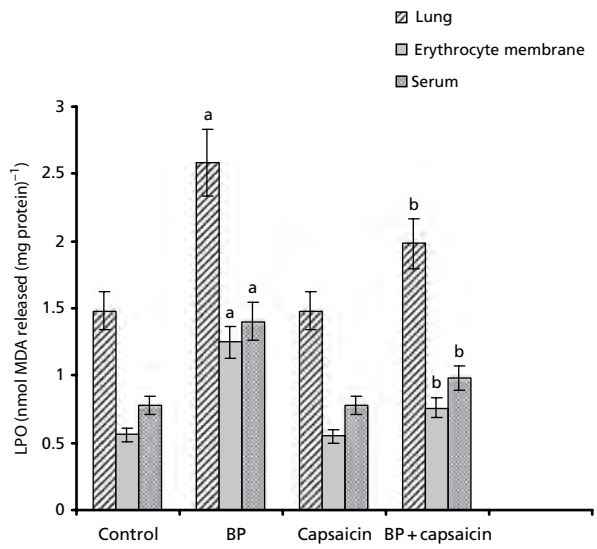

Figure 1 Levels of malondialdehyde (MDA) in tissue, erythrocyte membrane and serum of control and experimental mice. Results are expressed as mean ± s.d. for six mice in each group. [a]$P < 0.05$, statistically significant compared with Group 1 (Control); [b]$P < 0.05$, statistically significant compared with Group 2 (BP). LPO, lipid peroxidation; BP, benzo (a)pyrene.

Table 1 Effect of benzo(a)pyrene (BP) and capsaicin on the activity of ATPases in the erythrocyte membrane and in the lung of control and experimental mice

Parameter	Group 1 (Control)	Group 2 (BP)	Group 3 (Capsaicin)	Group 4 (BP + capsaicin)
Erythrocyte membrane				
Na^+/K^+ATPase	0.58 ± 0.04	0.17 ± 0.01[a]	0.47 ± 0.04	0.49 ± 0.05[b]
Ca^{2+}ATPase	0.51 ± 0.03	0.11 ± 0.01[a]	0.41 ± 0.04	0.45 ± 0.04[b]
Mg^{2+}ATPase	0.48 ± 0.03	0.10 ± 0.01[a]	0.47 ± 0.04	0.41 ± 0.04[b]
Lung				
Na^+/K^+ATPase	2.97 ± 0.28	1.76 ± 0.18[a]	2.97 ± 0.28	2.81 ± 0.29[b]
Ca^{2+}ATPase	2.23 ± 0.22	1.66 ± 0.16[a]	2.22 ± 0.22	2.12 ± 0.21[b]
Mg^{2+}ATPase	2.53 ± 0.26	1.73 ± 0.17[a]	2.51 ± 0.25	2.28 ± 0.23[b]

Results are expressed as mean ± s.d for six mice in each group. Na^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase units are μmol of inorganic phosphate formed min^{-1} (mg protein)$^{-1}$. [a]$P < 0.05$, statistically significant compared with Group 1; [b]$P < 0.05$, statistically significant compared with Group 2.

(Group 2). Capsaicin pretreatment resulted in a free radical quenching effect, thereby significantly ($P < 0.05$) preventing the peroxidation of lipids in Group 4 animals.

Table 1 shows the effect of BP and capsaicin on the activities of ATPases in erythrocyte membrane and lung tissues of control and experimental animals. A significant ($P < 0.05$) decrease in the activities of Na^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase was observed in lung cancer bearing animals (Group 2). These adverse effects were reversed to near normal in capsaicin-treated animals (Group 4).

Figure 2 shows the lung and total serum protein content in control and experimental animals. There was a significant ($P < 0.05$) decrease in the total protein levels in lung cancer bearing animals (Group 2) and pre-co-treatment with capsaicin markedly ($P < 0.05$) increased the total protein levels in Group 4 animals.

Table 2 shows the levels of tissue glycoproteins in control and experimental animals. BP treatment markedly ($P < 0.05$) increased the levels of glycoproteins sialic acid, hexose, hexosamine, hexuronic acid and fucose in Group 2 animals; these levels were significantly ($P < 0.05$) restored to near normal in capsaicin-pretreated animals (Group 4).

The influence of capsaicin on the levels of serum glycoproteins in control and experimental animals is shown in Figure 3. Lung cancer bearing animals showed a significant ($P < 0.05$) increase in the levels of the serum glycoproteins sialic acid, hexose, hexosamine, hexuronic acid and fucose. Pretreatment with capsaicin significantly ($P < 0.05$) reduced the levels of these glycoproteins to near normal levels in Group 4 animals.

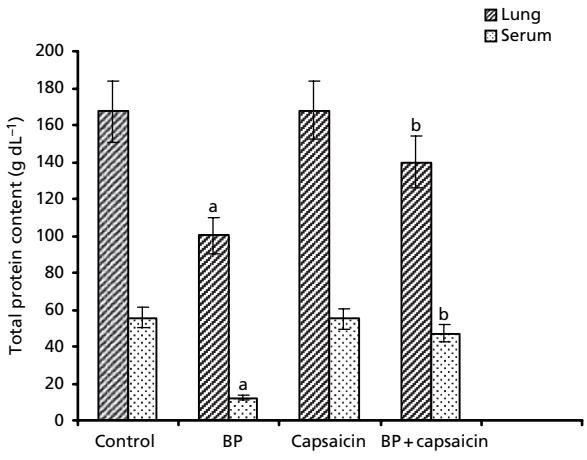

Figure 2 Total protein content in lung and serum of control and experimental mice. Results are expressed as mean ± s.d. for six mice in each group. [a]$P < 0.05$, statistically significant compared with Group 1 (Control); [b]$P < 0.05$, statistically significant compared with Group 2 (BP).

Discussion

Numerous studies have indicated that lung cancer is not the result of a sudden transformation in the bronchial epithelium, but rather a multi-step accumulation of genetic and epigenetic alterations. These are mostly caused by chronic exposure to carcinogens such as BP, which are predominantly present in tobacco smoke and automobile exhaust fumes, either actively or passively (Minna 1989; Thiberville et al 1995; Virmani et al 1998). Therefore, a strategy to arrest or reverse preneoplastic changes in the bronchial epithelium by natural or synthetic agents before invasive cancer develops is a rational approach for reducing the burden of lung cancer (Hu & Cassano 2000).

Progressive cellular architectural changes, as a result of oxidative stress, LPO and modulation of various cellular molecular pathways by reactive free radicals generated during cytochrome P450-dependent metabolism of BP, have been implicated in the pathogenesis of lung carcinogenesis (Das et al 2007). MDA formed by LPO is a highly reactive electrophile, capable of interacting with DNA to form MDA–DNA adducts that induce frame shifts and base-pair substitution mutation, leading to carcinogenesis (Ramakrishnan et al 2007). In the present study, we observed an increase in lung, erythrocyte membrane and serum MDA levels in BP-induced lung cancer animals. Capsaicin supplementation markedly reduced MDA levels, resulting in a free radical quenching

Table 2 Effect of benzo(a)pyrene (BP) and capsaicin on lung glycoprotein levels in control and experimental mice

Parameter	Group 1 (Control)	Group 2 (BP)	Group 3 (Capsaicin)	Group 4 (BP + capsaicin)
Sialic acid	44.8 ± 4.5	89.2 ± 9.1[a]	44.2 ± 4.4	52 ± 5.0[b]
Hexose	186 ± 19.2	271 ± 27.7[a]	185 ± 19.0	202 ± 20.2[b]
Hexosamine	33 ± 3.24	72 ± 7.12[a]	34 ± 3.35	41 ± 3.9[b]
Hexuronic acid	16.4 ± 1.7	58 ± 6.14[a]	16.1 ± 1.6	21 ± 2.12[b]
Fucose	9.3 ± 0.88	51 ± 5.27[a]	9.27 ± 0.92	13 ± 1.31[b]

Results are expressed as mean ± s.d. for six mice in each group. Sialic acid, hexose, hexosamine, hexuronic acid and fucose units are mg (g of defatted tissue)$^{-1}$. [a]$P < 0.05$, statistically significant compared with Group 1; [b]$P < 0.05$, statistically significant compared with Group 2.

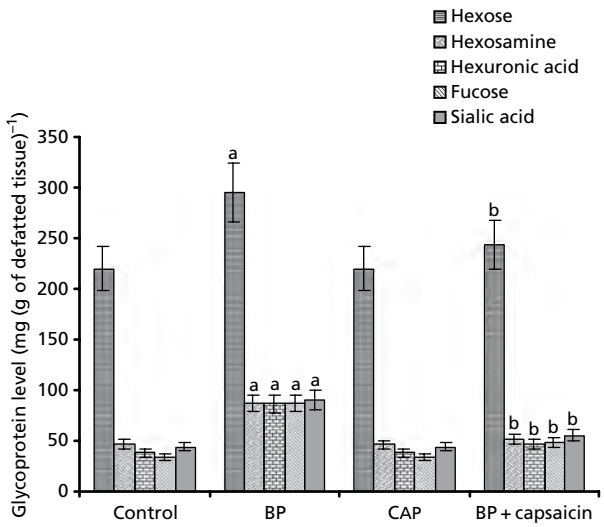

Figure 3 Serum glycoprotein levels in control and experimental mice. Results are expressed as mean ± s.d. for six mice in each group. [a]$P < 0.05$, statistically significant compared with Group 1 (Control); [b]$P < 0.05$, statistically significant compared with Group 2 (BP).

effect, signifying its potent antioxidant and anti-peroxidative effect.

Membrane-bound enzymes are sensitive indices of an altered cellular environment and could form one approach towards the understanding of the biochemical basis of the pathogenesis of diseases (Kempaiah & Srinivasan 2004). In malignancy, the cell membrane plays a crucial role in the stimulation and control of cell adhesiveness, mortality and proliferation in a damaged condition and, hence, protection of membranes is of potential importance in the treatment of disease processes (Thirunavukkarasu & Sakthisekaran 2003a). Oxidative stress is a predominant factor in lung cancer, causing membrane lipid oxidation, initiating loss of membrane-bound enzyme activity and cell lysis, and altering membrane permeability and cell function (Selvendiran & Sakthisekaran 2004). The protein moiety of Na^+/K^+ATPases is modified by free radicals and it is well known that as a result of oxidative stress, the membrane loses its component, resulting in increased deformability and cell lysis (Pandima Devi et al 2004). The reduced activity of Na^+/K^+ATPases indicates the changes in the membrane under pathological conditions (Rauchova et al 1995). Ca^{2+}ATPase is a reflection of energy-dependent calcium transport across the cell membrane. Decreased activity of this enzyme would contribute to the accumulation of intracellular calcium, which will then bind to the inner surface of the membrane and make the membrane less deformable (Benaim et al 1993). Mg^{2+}ATPase, along with the other ATPases, is also involved in energy-requiring processes in the cell. The metabolic products of BP generate free radicals and LPO in biological systems, which damage the membrane-bound enzymes and affect the normal functioning of the cell (Selvendiran & Sakthisekaran 2004). Our study is in agreement with the above finding: we observed reduced activity of all three membrane-bound ATPases, namely Na^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in BP lung cancer bearing animals. Capsaicin treatment returned the activities of these enzymes to near normal levels. The protective effects of capsaicin in this system could be either due to scavenging peroxides before attacking membrane and/or due to blocking the oxidation of membrane lipids.

Proteins play an important role in all processes in the body. Alterations in total protein content were observed in neoplastic tissues and a gross change in total protein levels takes place during the development of carcinogenesis (Selvendiran et al 2006). A decrease in total protein levels in cancer bearing animals has been previously reported (Thirunavukkarasu & Sakthisekaran 2003b). Our results are in agreement with the above findings as we observed decreased levels of total protein in lung cancer bearing animals. After capsaicin treatment, the total protein levels were markedly increased in Group 4 animals. This beneficial effect of capsaicin could be through quenching free radicals or reacting with the products of BP metabolites.

Glycoproteins exert a key role in mediating cell surface function, such as cell–cell recognition, cellular adhesion, binding and clearance of serum glycoproteins and metabolic transport, and they have been designed as non-specific markers of malignancy (Rachesky et al 1982). A change in surface carbohydrate moieties during cellular differentiation and neoplastic transformation suggests their importance in the physiology and behaviour of the cells (Hynes 1976). The crucial roles of cell surface and membrane constituents in neoplastic behaviour and changes in normal serum glycoconjugates have long been associated with malignancies (Olden et al 1982). The elevated serum glycoproteins are due to tissue necrosis,

rapidly metabolizing tumour, cell destruction from normal connective tissue and the non-specific stimulus in a process similar to that observed in many chronic infectious diseases (Lu et al 2000).

Sialic acid is an acylated derivative of neuraminic acid and exists as a terminal component of the non-reducing end of carbohydrate chains of glycoprotein in mammals. Their implications in a variety of surface-related vital cell functions in numerous tissues are well documented (Malarkodi et al 2003). Neoplasms often have an increased level of sialic acid on the tumour cell surface and the sialoconjugates are shed or secreted by some of these cells, which results in their increased levels in blood (Thirunavukkarasu & Sakthisekaran 2003b). Srinivasa Rao & Sirsi (1970) observed about 36% increase in the serum sialic acid levels in tumour bearing mice and hence the levels of sialic acid can be taken as a useful tool in the confirmation of tumours. The present observations also confirm the previous findings as we noticed increased tissue and serum sialic acid levels in lung cancer bearing animals. Administration of capsaicin remarkably reduced sialic acid levels to near normal values, suggesting its chemoprotective nature.

Increased levels of tissue and serum glycoproteins hexose, hexosamine, hexuronic acid and fucose during malignancy reflect either a local or systemic tissue response to tumour, which arises from the tumour itself (Nagini et al 1998). These findings agreed with our present observations where we noticed an increase in the tissue and serum levels of hexose, hexosamine, hexuronic acid and fucose in lung cancer bearing animals. Capsaicin pretreatment significantly reduced the levels of these glycoproteins to normal. This reduction in the levels of glycoprotein components indicates that capsaicin has the ability to suppress malignancy by modulating cellular transformation, signifying its chemoprotective function.

In conclusion, the results of the present study indicate that capsaicin supplementation alleviated the disruptions in LPO, membrane-bound enzyme activity, total protein and glycoprotein levels during BP-induced lung cancer, thus validating its chemopreventive effect.

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