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Inhibition of benzo[*a*]pyrene induced mutagenicity and genotoxicity by black tea polyphenols theaflavins and thearubigins in multiple test systems

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Abstract

This study investigated the antimutagenic and anticlastogenic effects of black tea polyphenols, theaflavins (TF) and thearubigins (TR) in Salmonella assay in vitro and in vivo in bone marrow cells of mice as measured by chromosomal aberrations (CA) and sister chromatid exchange (SCE) against a known carcinogen, benzo[*a*]pyrene (B[*a*]P). A significant decrease in mutagenicity in Salmonella assay and both CA and SCE were observed in all the different concentrations of TF and TR plus B[*a*]P treated series when compared with B[*a*]P treated group alone. These results indicate that both TF and TR have significant antimutagenic and anticlastogenic effects.

Keywords: Benzo[*a*]pyrene (B[*a*]P); Black tea (Tata Tea Gold); Theaflavins; Thearubigins; Antimutagenicity assay; Anticlastogenicity assay

1. Introduction

Tea is a highly consumed and popular beverage throughout the world. It is grown in over 30 countries exclusively in subtropical and tropical zones. According to its processing, tea can be classified in to green (20%), black (78%) or oolong (2%) tea. Black tea contains antioxidant substances, traces of proteins, carbohydrates, amino acids, lipids, and significant quantities of vitamins and minerals. It has been reported that tea extracts have antibacterial (Toda et al., 1989) antiviral (Nakayama et al., 1990), antioxidative (Matsuzaki and Hara, 1985), antitumor (Katiyar et al., 1993) antimutagenic (Weisburger et al., 1996; Yen and Chen, 1996) and anticarcinogenic (Goldbohm et al., 1996; Yang et al., 1997) effects. Weisburger et al. (1996) examined the effects of specific tea polyphenols (polyphenon 60 and polyphenon 100 from green tea and polyphenon B from black tea) against a number of genotoxic carcinogens in Salmonella strains TA98, TA100 and TA1535. Polyphenon B of black tea contains a mixture of polyphenols, mostly theaflavins (TF) and thearubigins (TR). These polyphenols sharply decrease mutagenicity of a number of aryl and heterocyclic amines of aflatoxin B1, B[*a*]P, 1-2 dibromomoethane and 2 Nitropropane, in the presence of an induced rat liver S9 fraction. Fresh tea leaves are rich in catechins and also contain polyphenol oxidase enzymes. During processing of tea leaves, catechins come in contact with polyphenol oxidase, joining them to one another to form TF and TR. During black tea manufacture most of the catechin mass is converted to

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a well defined group of compounds known as TR. Black tea contains major amount of these two polyphenols TR and TF which account for 12–18% and 3–6% of dry weight of black tea respectively. These polyphenols are responsible for the distinct color and flavor of black tea.

Most of the investigations have been carried out on green tea and its polyphenols whereas very little information is available about the antimutagenic and anticlastogenic effects of black tea polyphenols TF and TR. We have been working on the antimutagenic and anticlastogenic effects of black tea and its polyphenols in multiple test systems (Gupta et al., 2001; Gupta et al., 2002a,b). Considering the potent antimutagenic and anticlastogenic effects of green tea extracts against B[*a*]P, we recognized the need to extend our studies on the antimutagenic and anticlastogenic effects of black tea polyphenols TF and TR against B[*a*]P as measured by both Salmonella assay and CA and SCE assays in vivo in BALB/c mice. To avoid the batch-to-batch variation of black tea sold by the different retailers, we decided to carry out all the research work on a particular type of black tea (Tata Tea Gold) formulated by Tata Tea limited, which is one of the most popular tea available in the Indian market.

2. Materials and methods

2.1. Animals

Charles River male rats of 150–175 g. were used for the preparation of liver homogenate (S9) for bacterial antimutagenicity assays. They were kept four per cage with husk bedding. Laboratory bred BALB/c both male and female mouse, 10–12 weeks old, weighing 25–30 g were used for anticlastogenic experiments. They were kept 4 per cage with husk bedding. All the animals were received from the animal house of our institute and were fed balanced rodent pellet diet (Gold Mohar, Lipton Ltd., Chandigarh, India) and water ad libitum. The environment had a controlled 12 h light and 12 h dark cycle. Ambient temperature and relative humidity were 22 ± 2 °C and 55 ± 5% respectively.

2.2. Chemicals

Theaflavins and thearubigins were isolated from Tata tea Gold as described below, which was purchased from the local market. Biotin, histidine, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate, crystal violet, ampicillin trihydrate, tetracycline, benzo[*a*]pyrene (B[*a*]P), colchicine were supplied from Sigma Chemical Company (St. Louis, MO); agar and nutrient broth were supplied from Hi Media laboratories Ltd (India); 5-bromodeoxyuridine (BrdU) tablets (50 mg each) were purchased from the Boehringer Mannheim Biochemicals (Germany).

2.3. Bacterial strains

For antimutagenicity assays, Salmonella strains TA97a, TA98, TA100 and TA102 were used. These strains were provided by Dr. Bruce N. Ames, Biochemistry Division, University of California, Berkeley, USA. TA97a and TA98 detect frame shift mutagens, TA100 detects base pair mutagens. Maron and Ames (1983) primarily recommended both TA98 and TA100 for routine mutagenicity assay. TA102 contains A–T base pairs at site of the mutation (determined by DNA sequence analysis) in contrast to the other Salmonella tester strains that detect mutagens damaging the G–C base pair. In this tester strain the mutation has been introduced into a multicopy plasmid as a result around 30 copies of the mutant gene exist for back mutation (Levin et al., 1982).

2.4. Preparation of S9 fraction

The procedure of Ames et al. (1973) and Garner et al. (1972) were used for the preparation of rat liver homogenate (S9). Charles river male rats of 150–175 g were fed 0.1% phenobarbital in their drinking water for seven days. On day 6, no foods were provided for these rats. The next day, they were killed for the rat liver homogenate (S9). S9 mix was prepared following the method of Maron and Ames (1983). All steps of this preparation were performed at 0–4 °C with cold and sterile solutions and glassware. S9 fractions were distributed in 2 ml aliquots in small sterile plastic tubes, quickly frozen and stored at −80°.

2.5. Isolation of theaflavins (TR) and thearubigins (TR) from black tea

Both TF and TR were extracted from the black tea (Tata tea Gold). TF and TR were isolated according to the procedure of Xie et al. (1993). This extraction was carried out in the Medicinal Chemistry Department of our Institute. In brief, black tea (10 g) was extracted by boiling with water (250 ml), the aqueous fraction further extracted with chloroform to remove caffeine and successively with ethyl acetate and n-butanol by liquid–liquid partition (Roberts, 1962) Ethyl acetate fraction contains TF and unconverted catechins (−) epicatechin gallate: 6.9%; (−) epigallocatechin gallate: 7.7%, where as, the n-butanol fraction contains TR (Xie et al., 1993).

2.6. Selection of doses

The doses of TF and TR for antimutagenicity assays in bacterial systems were selected based on the percentage of these two polyphenols present in black tea. Black