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L-519, A PHENOLIC COMPOUND, INHIBITS METABOLISM OF BENZO(a)PYRENE AND MUTAGENESIS INDUCED BY BENZO(a)PYRENE

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L-519 is a phenolic compound. In this study, L-519 was found to inhibit the mutagenicity of benzo(a)pyrene [B(a)P] in Salmonella Typhimurium TA 97 and TA 100 and inhibit the cytochrome P-450 mediated metabolism of B(a)P. It also inhibited the epidermal ODC activity induced by croton oil. L-519 also decreased the lipid peroxidation induced by $FeSO_4$ and Cysteine in rat liver microsome system. Our results demonstrated that L-519 exhibited anti-mutagenicity, anti-initiation as well as anti-promotion activities.

Key words: Phenolic compound, Inhibition, Anti-mutagenesis, Anti-carcinogenesis, Benzo(a)pyrene.

The search for anti-carcinogenic agents and the understanding of the mechanisms of action are of critical importance in chemoprevention of cancer. In the search for effective inhibitors of carcinogenesis, both synthetic and naturally occurring compounds are being investigated. Fruits, vegetables, such as cabbage, cauliflower, garlic, onion, green coffee beans, green tea and traditional Chinese medicine provide a rich source of naturally occurring anti-mutagenic and anti-carcinogenic agents. Synthetic compounds such as retinoids, tamoxifen, oltipraz and N-acetylcysteine area group of well-known anti-mutagenic and anti-carcinogenic agents.[1-7]

The polyphenols are a large group of naturally occurring and synthetic compounds which are largely plant originated. Tannic acid is an antioxidant that is effective inhibiting late-stage tumor promotion elicited by phorbol esters.[8] A substantial number of experiments indicated that the polyphenols can decrease the mutagenicity and carcinogenicity of a wide variety of chemicals that include polycyclic aromatic hydrocarbons, aflatoxin B, and N-methyl-N-nitro-N-nitrosoguanidine.[9] Ellagic acid could inhibits benzo(a)pyrene metabolism and activation, and scavenges the activated diol epoxide. Ellagic acid is an excellent inhibitor of lipid peroxidation and relatively resistant to destruction by single oxygen, superoxide and γ-irradation.[10] As a phenolic compound, in this study, we studied the activity of L-519 on mutagenesis and carcinogenesis.

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MATERIALS AND METHODS

L-519 was provided by Dr. LN Lee, Department of Phytochemistry, Institute of Materia Medica, CAMS. L-

Ornithine, Phridoxal 5-Phosphate, NADPH were purchased from Sigma Chemical Co., Benzo(a)pyrene [B(a)P] were purchased from Fluka Chemical Co., B(a)P and L-519 were dissolved in DMSO. ^{3}[H] B(a)P (56.5 Ci/mMol) was purchased from Searle. Salmonella Typhimurium strains TA 97 and TA 100 are gifts from Professor BN Ames.

Anti-mutagenesis Assay

The mutagenicity assay was conducted according to Ames et al..[11] Salmonella Typhimurium strains TA 97 and TA 100 were used and with β-naphthoflavone (β-NF) and Phenobarbital (PB)-induced rat liver 9000 × g supernatant (S-9) was used as the enzyme source for metabolic activation.

B(a)P Metabolism Assay

B(a)P metabolism assay was carried out according to Chae. YH.[12] B(a)P metabolism was measured in S-9 rat liver homogenate induced by β-NF combined with PB. The incubation systems contained S-9 fraction, NADPH and various concentration of the test drugs (triplicate per dose) or DMSO. The reactions were started by the addition of ^{3}[H] B(a)P dissolved in acetone. After incubation, reactions were stopped by adding ice-cold methanol and chloroform. The organic and aqueous phase were then separated by centrifugation, and the radioactivity in both phases was determined by scintillation counter.

Ornithine Decarboxylase (ODC) Activity

ICR mice (6–8 weeks ♀) were pretreated with a single dose of L-519 (0.28 mM, 0.56 mM, 1.12 mM in o.2 ml of 0.9% NaCl) for 3 days. On the third days, one hour after the above pretreatment, the animals received a single topical application of 1% croton oil 0.2 ml in acetone. All animals were killed 5 h after croton oil treatment, and epidermis was separated by a brief heat treatment for 30 s at 52°C and 3000 × g centrifugation for 30 s and the supernatant was pre-pared[13] Enzyme activity was determined by measuring the release of $^{14}CO_2$ from L-(^{14}C) ornithine.[14] Enzyme activity was expressed as pmol CO_2/60 min/mg protein.

Lipid Peroxidation

The peroxidation of lipid was induced by adding 1 mM $FeSO_4$ and 10 mM Cysteine in rat liver microsome fraction. The incubations were carried out at 37°C for 30 min in a reaction mixture containing 10 mM phosphate buffer (pH 7.4) and test compound in a total volume of 1.0 ml. The reaction was terminated by the addition of 0.3 ml of 20% TCA. Thiobarbituric acid (0.6 ml, 0.67% TBA) was added, boiled for 10 min and after cooling the tubes under running tap water the absorbance at 532 nm was measured.

RESULTS

Effect of L-519 on Mutagenesis of TA 97 and TA 100

The antimutagenic activity of L-519 was evaluated in S. typhimurium strains TA 97 and TA 100 in the presence of rat liver S-9 as the enzyme source for the metabolic activition. B(a)P was used as promutagens. The data shown in Table 1 indicated that L 519 could inhibit the mutagenicity of B(a)P significantly.

Effect of L-519 on B(a)P Metabolism by S-9 Liver Fraction

The incubation of rat liver homogenate and NADPH in the absence of test compound metabolized 0.34 n mol of ^{3}[H] B(a)P to water-soluble derivatives in 10 min in control group. The addition of L 519 to the incubation mixtures resulted in a dose dependent decrease of B(a)P metabolism (Figure 1). It was demonstrated that L-519 could inhibit B(a)P metabolism by rat hepatic microsome induced by β-NF and PB.

Effect of L-519 on ODC Activity

As a rate-limiting enzyme in polyamine biosynthesis, the induction of ODC is one of the most important and characteristic biochemical parameters of croton oil-induced tumor promotion.[15,16] In our study, a single dose of L-519 for 3 days exhibited 30–70%