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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%

inhibition of epidermal ODC induced by croton oil in a dose-dependent manner (Table 2).

Effect of L-519 on Lipid Peroxidation

MDA contents were increased in rat liver microsome fraction after the addition of 1 mM FeSO and 10 mM Cysteine. It was shown that L-519 could significantly inhibit the lipid peroxidation in a dose-dependent manner (Table 3).

Table 1. Antimutagenic activity of L-519 in S. typhimurium TA 97 and TA 100

Group	Concentration (mg/plate)	His revertants TA 97	Inhibition (%)
Control		648± 41	
L-519	0.2	484± 10	25.39
	0.4	417± 7	35.77
		TA 100	
Control		600± 15	
L-519	0.2	423± 4	29.55
	0.4	395± 6	34.11

Each value represents $\bar{x}\pm s$ of triplicate.

Table 2. Effect of L-519 on epidermal ODC induction caused by croton oil

Group	Concentration (mM/kg)	ODC activity (pmol CO_2/60min/mg prot)	Inhibition (%)
Control		72.96± 28.38	
Croton oil		1492.55± 308.92	
L-519 + croton oil	0.28	973.52± 317.87*	34.77
	0.56	508.05± 111.12*	65.96
	1.12	403.64± 40.66*	72.97

* $P<0.01$ Each value represents $\bar{x}\pm s$. Five animals were pooled for each determination.

Table 3. Effect of L-519 on MDA content in rat liver microsome

Group	Concentration (μ M)	Lipid peroxidation (n mol MDA/mg protein)	Inhibition (%)
Control		6.21± 0.67	
L-519	60	4.44± 0.25*	28.50
	300	2.60± 0.44*	58.13

* $P<0.05$ Each value represents $\bar{x}\pm s$.

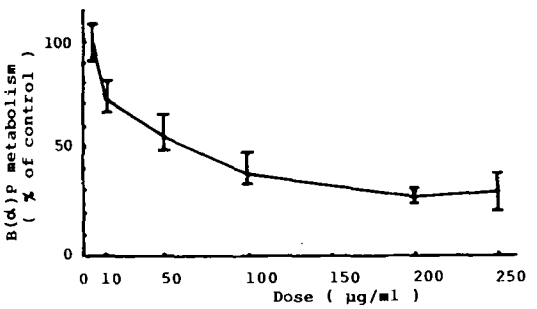

Fig. 1. Effect of L-519 on the metabolism of B(a)P by liver S-9 fraction from rats. The proportion of ^{3}[H] B(a)P converted to water-soluble derivation was determined by organic-solvent extraction and liquid scintillation counting. Each point represents the $\bar{x}\pm s$ of at least three determina- and is expressed as a percentage of the DMSO-treated control metabolism.

DISCUSSION

The polyphenols, such as tannic acid, ellagic acid, ellagitannins, are present in a fruit, nuts, vegetables and Geranium, Rhus, Acer plants.[17] This group of compound could inhibit the metabolic activation of carcinogens into molecular species that elicit DNA damage. They also act as scavengers of DNA-reactive metabolites of carcinogens such as benzo(a)pyrene, and exhibit antioxidant activity.[18, 19] L-519 is a phenol compound. Structurally, it belongs to a polyphenol synthetic analogue. In this study, L-519 was shown to have antimutagenic activity and inhibit B(a)P metabolism. The cytochrome P-450 dependent monooxygenase system catalyze oxidative metabolism of a wide variety of substrates. As a preliminary detoxification step, many xenobiotics are converted to polar metabolites by cytochrome P-450, which facilitates their elimination. However, some compounds may be inadvertently bioactivated by P-450 to reactive intermediates that produce adverse biological effects.[20] Benzo(a)pyrene undergoes metabolic activation by cytochrome P-450 and epoxide hydrase to chemically reactive ultimate carcinogenic diol epoxides.[21, 22] β-NF has been shown to induce cytochromes P-450IA$_1$ (P 450c) and P-450IA$_2$ (P-450d) in rat liver.[23] PB is a classical inducer of rat liver cytochromes P-450IIB (P-450b) and P-450IIB$_2$ (P-450e).[23-25] Cytochrome P-450IA$_1$, the major form of cytochrome P-450 present in the liver of rats treated with β-NF or PAH has high B(a)P metabolizing capacity.[25] L-519 inhibits B(a)P metabolism activation in S-9 rat liver homogenate induced by β-NF combined with PB. It is suggested that L-519 mainly inhibit P-450IA$_1$.

It was reported that some anti-inflamatory agents can prevent the skin tumor promotion.[26] In addition to the antipromotion activity in ear edema assay, we have also found that L-519 can inhibit the induction of ODC. It is suggested that L-519 has the antipromotion activity and could potentially be used as a cancer chemopreventive agent.

REFERENCES

1. Sparnins VL, Barany G, Wattenberg LM. Effects of organosulfur compounds from garlic and onions on benzo(a)pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. Carcinogenesis 1988; 9:131
2. Wang ZY, Khan WA, Bicker DR, et al. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. Carcinogenesis 1989; 10:411.
3. Wang ZY, Cheng SJ, Zhou ZC, et al. Antimutagenic activity of green tea polyphenols. Mutat Res 1989; 223:273.
4. Cassady JM, Baird WM, Chang CJ, et al. Natural products as a source of potential cancer chemotherapeutic and chemo-preventive agents. J Nat Pro 1990; 53(1):23.
5. Wei M, Tye L, Bresnick E, et al. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarbo-xylase and skin tumor promotion in mice. Cancer Res 1990; 50:499.
6. Hartman PE, Shankel DM. Antimutagens and anticarcinogens: A survey of putative interceptor molecules. Envir Mol Mut 1990; 15:145.
7. Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res (Suppl) 1992; 52:2085s.
8. Kensler TW, Kaotoh H, Tsutsui H, et al. Modification of aflatoxin B binding to DNA *in vivo* in rats fed phenolic

antioxidants and dithiolthione. Carcinogenesis 1985; 6: 759

9. Sayer JM, Yagi H, Wood AW, et al. Extremely facile reaction between the ultimate carcinogen benzo(a)pyrene 7, 8- diol- 9, 10- epoxide and ellagic acid. J Am Chem Soc 1982; 104:5562.
10. Nagabhushan M, Baumaun J, Bruchhausen FV, et al. Anti-mutagenicity of catechin against environmental mutagens. Mutagenesis 1988; 3:293.
11. Ames BN, McCann J, Yamasaki E. Methods of detecting carcinogens and mutagens with the salmonella mammalian microsome mutagenicity test. Mutat Res 1975; 31:347.
12. Chae YH, Marcus CB, Ho DK, et al. Effects of synthetic and naturally occurring flavonoids on benzo(a)pyrene metabolism by hepatic microsomes prepared from rat treated with cytochrome P-450 inducers. Cancer Lett 1991; 60:15.
13. Das M, KHan WA, Asokan P, et al. Inhibition of polycyclic aromatic hydrocarbon-DNA adduct formation in epidermis and lungs of SENCAR mice by naturally occurring plant phenols. Cancer Res 1987; 47:767.
14. Verma AK. Inhibition of phobol ester-caused synthesis of mouse epidermal ornithine decarboxylase by retinoic acid. Biochem Biophys Acta 1985; 846:109.
15. O'Brien TG. The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. Cancer Res 1976; 36:2644.
16. Athar M, Khan WA, Mukhtar H, et al. Inhibition of benzoyl-peroxide-mediated tumor promotion in DMBA initiated skin of SENCAR mice by antioxidants nordihydroguaiaretic acid and diallyl sulfide. J Invest Dermatol 1990; 94:162.
17. Takuo Okuda, Takashi Yoshida, Tsutomu Hatano, et al. Ellagitannins as active contituents of medicinal plants. Planta Medica 1989; 55:117.
18. Gary DS, Mark AM. Newer agents: drugs vs natural products. 4th Inter. Conf. Prev. Human Cancer: Nutri. Chemoprev Contrav. Arizona. 1992; June 3– 6.
19. Wall ME, Wanb MC, Manlkumar G, et al. Plant anti-mutagenic agents, 2. Flavonoids. J Nat Prod 1988; 51:1084.
20. Gelboin Hv. Benzo(a)pyrene metabolism, activation and carcinogenesis; role of regulation of mixed function oxidases and related enzymes. Physiol Rev 1980; 60:1107.
21. Conney AH. Inhibition of microsomal enzymes by foreign chemical and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. Cancer Res 1982; 42:4875.
22. Guengerich FP, Dannan GA, Wright ST, et al. Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic and immuno-chemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-naphthoflavone. Biochem 1982; 20:6019.
23. Lau PP, Strpbel HW. Multiple of forms of cytochrome P-450 in liver microsomes from β-naphthoflavone-pretreated rats. Separation, Purification and Characterization of five forms. J Biol Chem 1982; 257:5257.
24. Waxman DJ, Walsh C. Phenobarbital-induced rat liver cytochrome P-450. Purification and Characterization of two closely related isozymic forms. J Biol Chem 1982; 257:10446.
25. Buening MK, Chang RL, Huang MT, et al. Activation and inhibition of benzo(a)pyrene and aflatoxin B metabolism in human liver microsomes by naturally occurring flavonoids. Cancer Res 1981; 41:67.
26. Gschwendt M. The mouse ear edema: a quantitatively valuable assay for tumor promoting compounds and inhibitors of tumor promoting. Cancer Lett 1984; 25:177.