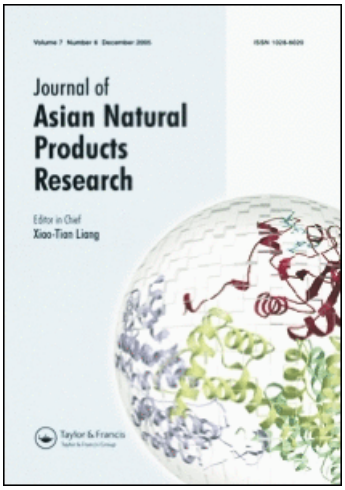

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Cancer Chemopreventive Activities of S-3-1, A Synthetic Derivative of Danshinone

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CANCER CHEMOPREVENTIVE ACTIVITIES OF S-3-1, A SYNTHETIC DERIVATIVE OF DANSHINONE

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Salvia miltiorrhiza is a traditional Chinese medicine which has been well documented for its anti-cancer effects. Based on the structure of danshinone, one of the active compounds derived from *Salvia miltiorrhiza*, we synthesized a simplified phenolic analog, S-3-1, and tried to explore its possible actions in preventing the development of cancer. With the Ames test, S-3-1 was found to efficiently suppress the mutagenicity of benzo[α]pyrene. This result is consistent with the inhibitory effect of S-3-1 on the activation of benzo[α]pyrene by hepatic microsomal enzymes. Besides the anti-initiation effects, S-3-1 could significantly inhibit the croton oil-induced increase of mouse skin epithermal ornithine decarboxylase activity. Moreover, S-3-1 quenched both superoxide and hydroxyl free radicals whereas it inhibited lipid peroxidation in the *in vitro* model. These results suggest that S-3-1 might act as anti-initiation and anti-promotion agents through reversing the biochemical alterations induced by carcinogen during carcinogenesis. Therefore, we further investigated the effects of S-3-1 on carcinogenesis. *In vitro*, S-3-1 inhibited the benzo[α]pyrene-induced transformation of V79 Chinese hamster lung fibroblasts. At 10 40 mg/kg, S-3-1 was found to inhibit the development of DMBA/croton oil-induced skin papilloma in mice through decreasing the incidence of papilloma, prolonging the latent period of tumor occurrence and reducing tumor number per mouse in a dose-dependent manner. We concluded from this study that S-3-1 might be developed as a new chemopreventive drug.

Keywords: *Salvia miltinorrhiza*; Danshinone derivative; Carcinogenesis; Chemoprevention; Anti-cancer drug

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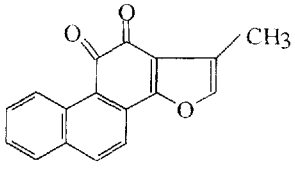
INTRODUCTION

Salvia miltiorrhiza is a classical traditional Chinese medicine which has been widely used for thousands of years for the treatment of cardiovascular diseases and inflammation in China. Recently, increasing evidence suggests that *Salvia miltiorrhiza* might be beneficial to cancer patients. After feeding the extracts from *Salvia miltiorrhiza*, the mice bearing Avery carcinoma survived longer. On the other hand, this medicine was reported in the animal models to enhance the anti-cancer effect of cyclophosphamide or camptothecine on a large variety of cancers, such as Avery carcinoma, lung carcinoma, S-180 sarcoma, etc. [1].

Danshinone(**I**) is one of the active components derived from *Salvia miltiorrhiza*. Its anti-cancer activities were well documented. Based on its structure, a series of simplified analogs were synthesized. S-3-1 is one of these compounds with strong inhibitory effect on the growth of cancer cells. After extensive investigation of the pharmacological characteristics of this compound, we found S-3-1 may be useful to prevent cancer development and outgrowth. In this study, to explore the possibility of developing S-3-1 as a new anti-cancer drug, we investigated the effects of S-3-1 on benzo[α]pyrene-induced mutagenesis of Salmonella bacteria, activation of latent mutagen, croton oil-induced increase of ornithine decarboxylase activity and oxidative damage. We also report here its effects on chemically-induced cellular transformation and DMBA/croton oil-induced murine skin papilloma.



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RESULTS AND DISCUSSION

Effect of S-3-1 on B[α]P-induced Mutagenesis in Salmonella Bacteria

TA97 and TA100 are two strains of the His$^-$ mutations of *S. typhimurium*. As a potent mutagen, S_9-activated benzo[α]pyrene induced mutation of these two strains back to the His$^+$ phenotype. When S-3-1 was included in the culture medium of bacteria, the rate of mutation induced by B[α]P was

decreased (Tab. I). 0.4 mg of S-3-1 significantly inhibit the mutagenicity of B[α]P.

Effect of S-3-1 on Metabolism of B[α]P by S_9 Liver Fraction

B[α]P is a latent mutagen which can be activated by metabolism in the presentce of hepatic microsomal enzymes. When incubation with S_9 rate liver homogenate fraction and NADPH, B[α]P was catalyzed to metabolize to water-soluble derivative with an average rate of 0.034 nmol/min. The addition of S-3-1 into the incubation mixture resulted in the decrease of B[α]P metabolism rate in a dose-dependent manner (Fig. 1). This result suggests that S-3-1 may prevent the *in vivo* activation of latent mutagen such as B[α]P.

TABLE I Effect of S-3-1 on B[α]P-induced mutation of Salmonella TA97 and TA100 bacteria

	TA97		*TA100*	
Group	*No. of mutants*	*Inhibition* (%)	*No. of mutants*	*Inhibition* (%)
Control	648 ± 41		600 ± 15	
S-3-1				
0.2 mg/plate	484 ± 10	25.4	423 ± 4	29.6
0.4 mg/plate	417 ± 7*	35.8	395 ± 6*	34.1

*$P < 0.05$.

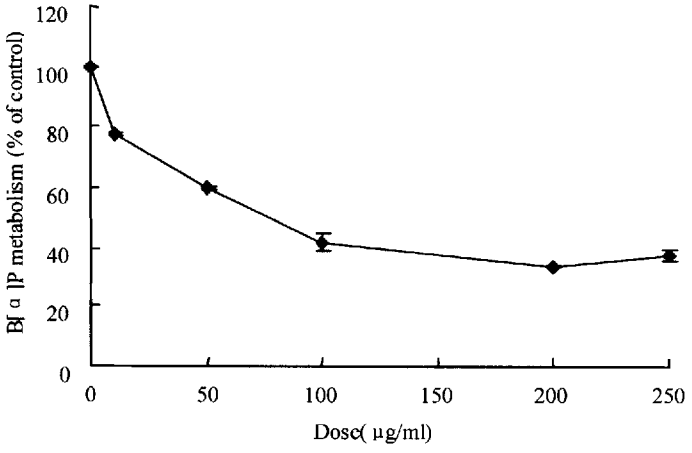

FIGURE 1 Effect of S-3-1 on the metabolism of B[α]P by rat hepatic microsome S_9 fraction.

Effect of S-3-1 on Croton Oil-promoted Epidermas Ornithine Decarboxylase Activity

As a rate-limiting enzyme in polyamine biosynthesis, ODC is believed to be associated with the outgrowth of the cells during promotion process in carcinogenesis. Croton oil, a reported tumor promotion agent, induced at least 20-fold increase of ornithine decarboxylase activity in epithelial cells. When the animals were administrated p.o S-3-1 once every day for 3 days, the croton oil-induced increase of epidermal ODC activity was decreased in a dose-dependent manner (Tab. II). The largest inhibitory rate could reach 73.0% for a dosage of 1.12 mmol/kg.

Effect of S-3-1 on Lipid Peroxidation

MDA contents were increased in microsome fraction from rat liver following the addition of 1 mM $FeSO_4$ and 10 mM cysteine. Table III shows that S-3-1 inhibit the lipid peroxidation significantly in a dose-dependent manner.

Scavenge of Free Radicals by S-3-1

ESR spectrum analysis is a rapid, simple and sensitive method to investigate the scavenge effects of drugs on free radicals. Figure 2A depicts that S-3-1 scavenges DPPH, the arbitrary synthesized free radical, in a

TABLE II Effect of S-3-1 on epidermal ODC activity induced by croton oil

Group	*ODC activity* (*pmol CO_2/60 min/mg*)	*Inhibition* (%)
Control	72.96 ± 28.38	
Croton oil	1492.55 ± 308.92	
Croton oil + S-3-1		
5 mg/kg	973.52 ± 317.87*	34.8
10 mg/kg	508.05 ± 111.12*	66.0
20 mg/kg	403.64 ± 40.66*	73.0

*$P < 0.01$.

TABLE III Effect of S-3-1 on lipid peroxidation

Group	*Concentration* (*μg/ml*)	*MDA content* (*nmol/mg*)	*Inhibition* (%)
Control		6.21 ± 0.67	
S-3-1			
	10	4.44 ± 0.25*	28.50
	50	2.60 ± 0.44*	58.13

*$P < 0.05$.

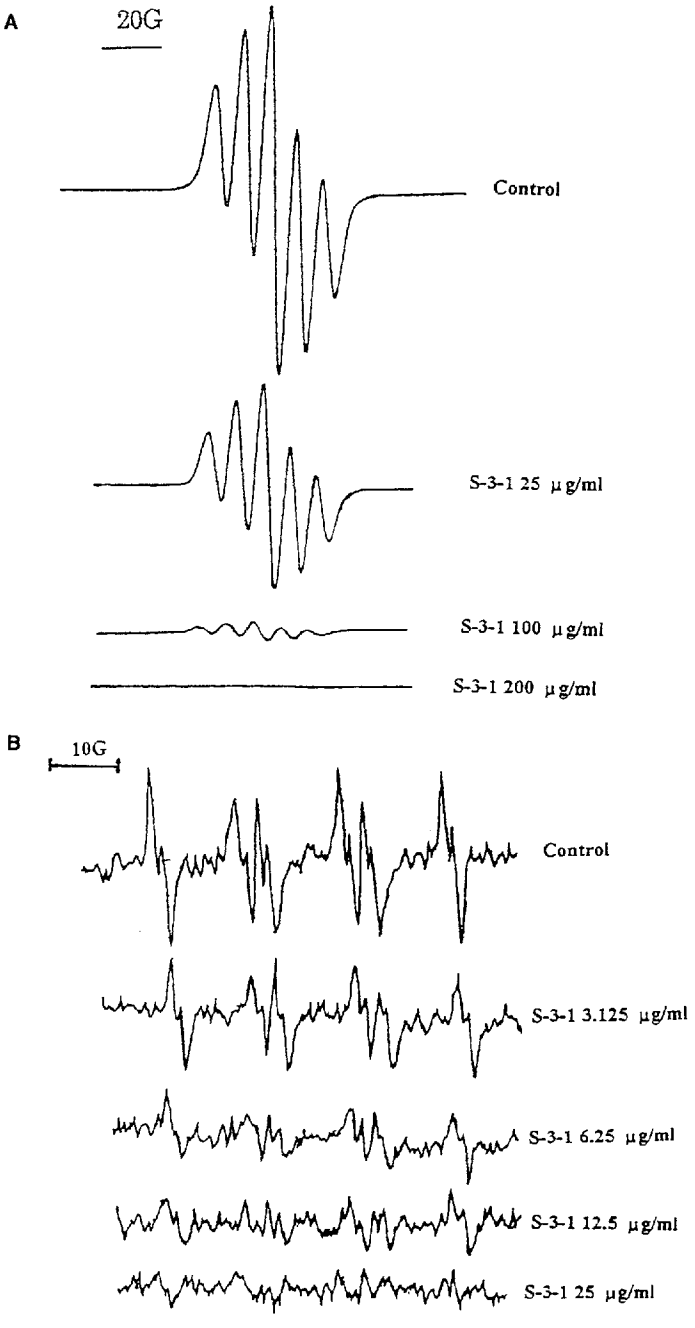

FIGURE 2 Effects of S-3-1 on electron spin resonance spectra of DPPH (A), superoxide free radical spin adduct (B), and hydroxyl free radical spin adduct (C).

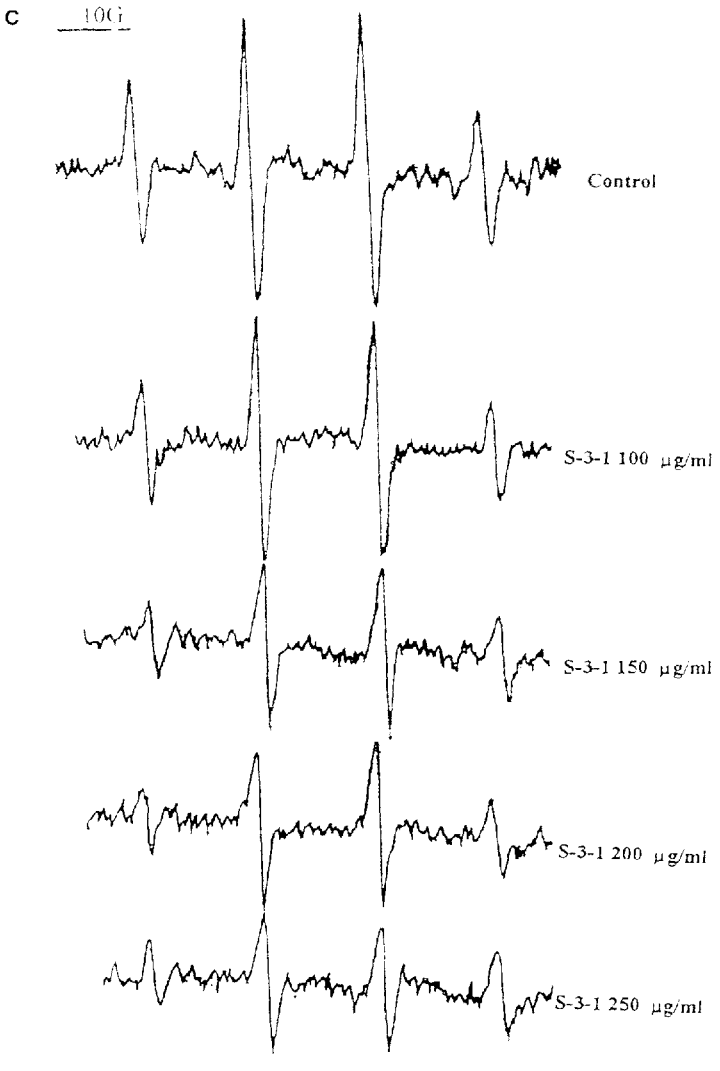

FIGURE 2 (Continued).

dose-dependent manner. Two hundred μg/ml of S-3-1 completely quenched DPPH radicals. Hypoxanthine transformation reaction catalyzed by xanthine oxidase is one of the ways to produce superoxide free radicals *in vivo*. With ESR analysis, S-3-1 at concentrations ranged from 3.125 to 25 μg/ml was found to be efficient to quench superoxide free radicals arisen from hypoxanthine/xanthine oxidase system (Fig. 2B). Twenty five μg/ml of S-3-1

completely quenched the superoxide free radicals. Similarly, at concentrations ranged from 100 to 250 μg/ml, S-3-1 also quenched the hydroxyl radicals produced by Fenton's reaction, though the scavenging extent was less than that of DPPH and superoxide (Fig. 2C).

Prevention of B[α]P-induced Cellular Transformation by S-3-1

S_9-activated B[α]P is a potent carcinogen that can induce the transformation of V79 cells. With the above described protocol, B[α]P induced the formation of a larger amount of countable transformation foci in the fibroblast monolayer. The number of transformation foci per dish was 27.67 ± 5.24. Contrary to the monolayer V79 cells, the cells isolated from the transformation foci can grow to form a colony in soft agar, suggesting the aggressive characteristic of these cells. The colony formation rate in soft agar is $167.25 \pm 5.67/10^4$ cells. However, when S-3-1 was added into the cell culture medium, B[α]P-induced formation of transformation foci was dramatically inhibited. Ten μg/ml and 20 μg/ml of S-3-1 decreased the number of transformation foci per dish to 11.33 ± 4.16 and 8.00 ± 3.25 respectively (Tab. IV). Moreover, the transformed cells from S-3-1-treated groups were shown a significant decrease in their ability of colonization in soft agar. The colony formation rates in soft agar were 60.25 ± 3.7 and $37.14 \pm 3.56/10^4$ cells for 10 μg/ml S-3-1-treated group and 20 μg/ml group respectively (Tab. IV).

Effect of S-3-1 on Skin Papilloma Formation Induced by DMBA/Croton Oil in Mice

By the fifth week of the experiment, the occurrence of skin papilloma was noted in the control group. Hereafter, the incidence of papilloma and the

TABLE IV Effect of S-3-1 on B[α]P-induced transformation of V79 Chinese hamster lung fibroblasts

Group	*B[α]P (μg/ml)*	*S-3-1 (μg/ml)*	*No. of transformation foci/10^4 cells*	*Colony formation rate/10^4 cells*
Control			3.43 ± 1.54	0
B[α]P	0.5		27.67 ± 5.24	167.25 ± 5.67
S-3-1 + B[α]P	0.5	1	26.54 ± 2.38	162.45 ± 4.89
	0.5	5	23.54 ± 2.38	$127.43 \pm 7.25^*$
	0.5	10	$11.33 \pm 4.16^*$	$60.25 \pm 3.17^*$
	0.5	20	$8.00 \pm 3.25^*$	$37.14 \pm 3.56^*$

$^*P < 0.01$ as compared to B[α]P group.

tumor number per mouse increased step by step (Figs. 3 and 4). By the end of the twelfth week, the incidence of papilloma was up to 92%, and the tumor number per mouse was up to 6.25. When the mice were given p.o. S-3-1 (10, 20, and 40 mg/kg) during the whole experiment process, the latent period of tumor occurrence was prolonged while the incidence of papilloma

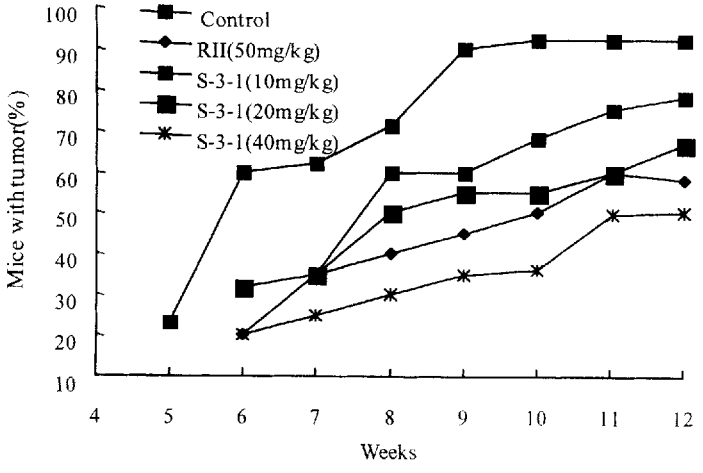

FIGURE 3 Effects of S-3-1 on DMBA/croton oil-induced skin papilloma in mice.

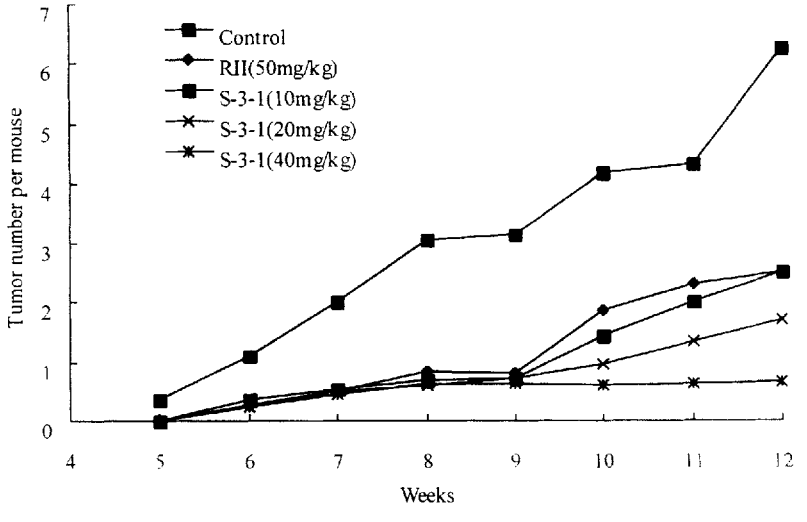

FIGURE 4 Effect of S-3-1 on tumor number per mouse induced by DMBA/croton oil.

was also decreased. The incidences of papilloma were 78%, 67% and 50%, respectively. the tumor number per mouse was also decreased in a dose dependent manner to 2.5, 1.7 and 0.67 for 10, 20, and 40 mg/kg of S-3-1 respectively (Figs. 3 and 4).

DISCUSSION

As an analog of the active component from *Salvia miltiorrhiza*, S-3-1 can be synthesized in a large scale due to its simple structure and small molecular mass. In this study, we tried to explore the possible actions of S-3-1 to prevent the development of cancer. According to a commonly-accepted theory, the development of cancer includes initiation, promotion and progression [8]. Reversion of the biochemical alternations caused by enviornmental factors, mutagen, for example, during these processes has been proved to be one of the activities of the chemopreventive compounds such as retinomide, selenites and carotenoids [9]. We thus firstly examine the effects of S-3-1 on mutagenesis, chmically-induced ODC activity, free radicals and other carcinogenesis-associated biochemical changes. B[α]P is a potent carcinogen which is strongly suspected to be associated with many human cancers when it is activated in the liver. With the Ames test model, S-3-1 was found to suppress B[α]P-induced mutation in the genomes of Salmonella bacteria. Accordingly, the metabolism of B[α]P by hepatic microsomal enzymes was inhibited by S-3-1. These results suggest that S-3-1 might prevent the mutation through suppression of the metabolized-activation of mutagen *in vivo* during the initiation of cancer development. Carcinogens such as croton oil and TPA may promote the development of cancer through extreme enhancement of the growth of the mutated cells. The outgrowth of epithelial cells is characterized by the increase of cellular ODC activity. As expected, S-3-1 suppressed croton oil-induced increase of mouse epidermal ODC activity, suggesting an alternative action of S-3-1 as a potent anti-promotion agent. Free radicals have been found to involve in both the initiation and promotion processes of carcinogenesis. It is widely accepted that active oxygen could cause lipid peroxidation, DNA damage and oncogene expression which thereby result in carcinogenesis [10]. As a phenolic compound, S-3-1 quenched both the superoxide, hydroxyl free radicals while it also inhibited the lipid peroxidation caused by Fe^{2+}/cysteine system. It is reasonably assumed that S-3-1 may scavenge free radicals arising from the metabolism of carcinogen and outgrowth of cells during carcinogenesis. Therefore, the above described results defined two actions of S-3-1, that is, anti-initiation

and anti-promotion, suggesting the possible chemopreventive actions of this compound.

Consistent with the actions of reversing the biochemical alternations during carcinogenesis, we found that S-3-1 prevented carcinogenesis in either an *in vitro* cellular transformation model or an *in vivo* chemically-induced carcinogenesis model. As a potent carcinogen, S_9-activated B[α]P induced transformation of V79 Chinese hamster lung fibroblasts. S-3-1 could significantly prevent the B[α]P-induced formation of transformation foci by V79 cells. In the S-3-1-treated groups, both the number of the transformed foci and the aggression of the transformed cells decreased dramatically. S-3-1 can also prevent carcinogenesis *in vivo*. When treated with S-3-1, the latent period of tumor occurrence was prolonged while the incidence of papilloma and tumor number were also decreased.

Chemoprevention of carcinogenesis is one of the efficient strategies to defeat cancer. Currently a large variety of compounds with different structure have been developed aiming at prevention of cancer development. Some of them have been undergoing the clinical trials and are expected to be approved by FDA in the near future [11]. S-3-1 is a novel compound with exciting chemopreventive actions described in this study. Our preliminary study showed that toxicity of S-3-1 is very low that the LD_{50} value is higher than 1 g/kg in mice. Moreover, in our other related studies, the effects of S-3-1 on the growth of cancers are also found. Therefore, we believe the possibility of developing S-3-1 as a novel anti-cancer drug.

EXPERIMENTAL SECTION

General Experimental Procedures

S-3-1, dissolved in water, was provided by the Department of Phytochemistry, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. Benzo[α]pyrene (B[α]P), 7,12-dimethyl benz[α] anthracene (DMBA), hypoxanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), DMPO, diethylenetriaminepentaacetic acid (DETAPAC) and xanthine oxidase were purchased from Sigma Chemical Co. [^{3}H]B[α]P was purchased from Dupont NEN Company. L-[^{14}C]ornithine was purchased from Amersham Searle Company.

Animals

Kunming mice and ICR mice were provided by the Animal Breeding Center of Chinese Academy of Medical Sciences.

Ames Test

Ames test was adopted to examine the effect of S-3-1 on B[α]P-induced mutagenesis in the Salmonella TA97 and TA100 bacteria [2]. Briefly, the bacteria were suspended in a soft agar mixture containing 10 μg/ml B[α]P (dissolved in DMSO), 0.5 ml S_9 and 0.2 mg/plate, 0.4 mg/plate of S-3-1, followed by layering on the agar plate. After 2-days incubation at 37°C, the bacteria colonies in each plate were counted.

Metabolism of B[α]P by Liver Homogenate S_9 Fraction

The reaction was performed as described [3] with a little modification. Buffered with phosphate salt (pH 7.4), the reaction mixture contained 1.2 mg S_9, 0.35 μmol NADPH, 20 μl DMSO or 10, 50, 100, 150, 200 and 250 μg/ml of S-3-1 with a total volume of 0.8 ml. [^{3}H] B[α]P (3.17 nmol) was added rapidly to the mixture while kept in dark. After incubation at 37°C for 10 min, 2 ml cold methanol and 1 ml chloroform were added to terminate the reaction. Following extraction with 1 ml chloroform and 1 ml water, dpm value in the organic phase or the water-soluble phase was determined by liquid scintillation to calculate the reversion ratio of B[α]P and its water-soluble metabolite.

Croton Oil-induced Epidermas Ornithine Decarboxylase Activity

ICR mice (18–22 g, male) were used in this experiment. All the animals were shaved followed by spreading of 200 μl of 1% croton oil (in acetone) on the skin of the mouse back. Five hours later, the animals were sacrificed and the back skins were sheared out. The skins were immersed into cold balanced salt followed by immersing in hot water (52°C) for 30 s. After being placed in cold balanced salt, the skins were scraped to obtain the epidermas layer. The activity of ornithine decarboxylase (ODC) in the epidermas homogenate was determined by the method described [4].

Fe^{2+}/Cysteine-induced Lipid Peroxidation

The lipid peroxidation was induced by adding 1 mM $FeSO_4$ and 10 mM cysteine in rat liver microsome preparation. After 30 min of incubation at 37°C, 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 the tubes were cooled under a running tap water. The absorbance at wavelength of 532 nm was measured and MDA was used as standard [5].

Electron Spin Resonance Spectrometry

Electron spin resonance (ESR) spectrometry was adopted to investigate the effects of S-3-1 on scavenging free radicals [6]. For DPPH free radical analysis, 10 μl of 25, 100 and 200 μg/ml of S-3-1 was added to 50 μl of 60 μmol DPPH (in ethanol), followed by vortexing and placing in the spectrometer flat cell to analyze after 60 s. For superoxide free radical analysis, 5 μl of 3.125, 6.25, 12.5 and 25 μg/ml of S-3-1 was mixed with 0.42 mmol/l hypoxanthine, 1.25 mmol/l DETAPAC, 80 mmol/l DMPO, and 0.07 U of freshly prepared xanthine oxidase suspension. DMPO-O_2 spin adducts were analyzed at 60 s after the addition of DMPO. For hydroxyl free radical analysis, 0.3 mmol/l $FeSO_4$, 0.3 mol/l H_2O_2, 80 mmol/l DMPO and 15 μl of 100, 150, 200 and 250 μg/ml of S-3-1 were mixed. The DMPO-OH spin adducts were analyzed at 60 s after addition of DMPO.

B[α]P-induced Transformation of V79 Chinese Hamster Lung Fibroblast

V79 Chinese hamster lung fibroblast cells were cultivated in RPMI 1640 medium supplemented with 10% calf serum. Before transformation, 1×10^4 V79 cells were inoculated into 25 cm^2 dish. Twenty-four hours later, B[α]P (0.5 μg/ml) and 20 μl S_9 were added and the cells were for 7 days. On day 8, B[α]P was washed away while the fresh culture medium was replenished. The cells were continuously cultivated until day 30 before treatment with Wright–Giemsa staining solution. Transformation foci were observed and counted under the inverse microscope. The transformation foci were defined as the up-piled cell population with abnormal shape and without growth contact inhibition. As a control, 0.1% DMSO was added into the culture medium instead of B[α]P. For drug treatment, 1, 5, 10 and 20 μg/ml of S-3-1 was also added into the culture medium to various final concentrations on day 2.

DMBA/Croton Oil-induced Mouse Skin Papilloma in Mice

The experiment was performed as described previously [7]. Briefly, 100 Kunming mice were divided into five groups of 20 each. After being shaved, all animals received a single topical application of DMBA (150 μg in 0.2 ml acetone) on days 1, 7 and 14, respectively. In the fifth week of the experiment, all animals received single topical application of croton oil (0.2 ml of 0.25% in acetone). This treatment was repeated twice weekly till the end of the experiment in the twelfth week. The animals in groups 3, 4, 5 received

S-3-1 (10, 20, 40 mg/kg) by p.o. route every other day, while mice in group 2 received N-4-(carboxyphenyl) retinamide (RII, 50 mg/kg) as positive control. The incidence of skin papilloma and the number of mice with tumors were recorded weekly over the entire period of the experiment.

References

[1] Liu, C. and Peng, M., *The Great Dictionary of Anti-cancer Chinese Medicine*. Human Science and Technology Press, Changsha, 1994, p. 210.

[2] Maron, D. M. and Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Research*, **113**, 173–215.

[3] Chae, Y. H., Marcus, C. B., Ho, B. K., Cassady, J. M. and Baird, W. M. (1991). Effects of synthetic and naturally occuring flavonoids on benzo[α]pyrene metabolism by hepatic microsomes prepared from rats with cytochrome p-450 inducers. *Cancer Letters*, **60**, 15–24.

[4] Gali, H. U., Perchellet, E. M. and Perchellet, J. P. (1991). Inhibition of tumor promoter-induced ornithine decarboxylase activity by tannic acid and other polyphenols in mouse epidermis *in vivo*. *Cancer Research*, **51**, 2820–2825.

[5] Bruch, R. C. (1983). Differential effect of lipid peroxidation on membrane fluidity as determined by electron spin resonance probes. *Biochimica et Biophysica Acta*, **733**, 216–223.

[6] Santiago, L. A., Hiramatsu, M. and Mori, A. (1992). Japanese soybean paste miso scavenges free radicals and inhibits lipid peroxidation. *Journal of Nutrition Science and Vitaminology (Tokyo)*, **38**, 297–304.

[7] Chen, X., Liu, H., Lei, X., Fu, Z., Li, Y., Tao, L. and Han, R. (1998). Cancer chemopreventive and therapeutic activities of red ginseng. *Journal of Ethnopharmacology*, **60**, 71–78.

[8] Farber, E. (1988). Cancer development and its natural history: A cancer prevention prospective. *Cancer*, **62**, 1676–1679.

[9] Greenwald, P., Nixon, D. W., Malone, W. F., Kelloff, G. J., Stern, H. R. and Witkin, M. (1990). Concepts in cancer chemoprevention research. *Cancer*, **65**, 1483–1490.

[10] Cerutti, P. A. (1985). Peroxidation states and tumor promotion. *Science*, **227**, 375–381.

[11] Greenwald P., Kelloff, G., Burch-Whitman, C. and Kramer B. S. (1995). Chemoprevention. *CA Cancer Journal of Clinics*, **45**, 31–49.