Arch. Pharm. Res. 12(2), 94-98 (1989)

Suppression of Diesel Emission Particle Extract-induced Micronuclei in Mouse Bone Marrow Cells by Pre-treatment with Ascorbic acid, α-Tocopherol or Glutathione

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(Received March 17, 1989)

Abstract□Induction of micronuclei by diesel emission particle extract (DEPE) in mouse bone marrow cells was suppressed by pre-treatment with ascorbic acid, α-tocopherol or glutathione (GSH). These compounds were given orally to mice at the dose of 1, 10 or 100 mg/kg/day for 5 consecutive days. The dose of DEPE (200 mg/kg) was administered intraperitoneally once to mice with the 5th administration of test compounds. Ascorbic acid, α-tocopherol and GSH all showed the dose-dependent suppression on DEPE-induced micronuclei.

Keywords□Anticlastogenic effect, Diesel emission particle extract, Micronucleus test, Ascorbic acid, α-Tocopherol, Glutathione.

In recent years many studies have become clear that a great number of mutagens and carcinogens exist in our environment.[1-4] Especially the potential carcinogenicity of diesel emissions has been an important problem in environmental carcinogenesis and/or mutagenesis.[5,6] Recent studies have indicated that exposure of the emissions of dieselled to lung cancers.[7,8] Such a diesel emission particles are distributed as large extents in our urban atmosphere.[9,10]

From the aspects, much attention has been focused on the finding of antimutagenic and/or anticarcinogenic factors that inactivate environmental mutagens or suppress the process of its genotoxic effects. Several compounds have been reported to exert inhibition on one or more mutagen(s) and/or carcinogen(s).[11,12] Ascorbic acid can suppress the *in vitro* and *in vivo* formation of nitrosoamines and nitrosoamides from nitrite, amines and amides.[13] α-Tocopherol can also suppress the formation of genotoxic nitroso compounds.[14]

According to Ito *et al.*,[15] various vegetable juices and glutathione showed the significant suppression of dimethyl benzo(a)anthracene-induced chromosome aberration. Inouye *et al.*[16] found that the induction of micronuclei by mitomycin C in mouse bone marrow cell was suppressed by post-treatment with vanillin.

In the present study, several biologically active compounds, such as ascorbic acid, α-tocopherol and glutathione, were tested for anticlastogenicity toward diesel emission particle extract (DEPE) having potential mutagenicity and/or carcinogenicity in the mouse bone marrow micronucleus test. It was found that micronuclei induced by DEPE in mouse bone marrow cells were significantly suppressed by pre-treatment with ascorbic acid, α-tocopherol or glutathione.

EXPERIMENTAL METHOD

Sampling and extraction of diesel emission particles

Diesel emission particles deposited on the inner wall of an exhaust pipe of a diesel bus were extracted by soxhlet apparatus using the benzene-ethanol (3:1) solution for 24 h. The extracts were filtered, evaporated by rotary evaporator and stored at -20 °C. The percent composition of tar in diesel emission particles was found to be approximately 1 percent. DEPE dissolved in dimethylsulfoxide was administered by intraperitoneal injection.

Animals

ddY Mice, 6 weeks old and weighing 28 ± 2 g, were supplied from the experimental animal breeding center in college of pharmacy, Kangweon Na-

Table I. The frequency of MNPCE after a single injection of DEPE at 300 mg/kg, i.p. into male and female ddY mice

Sampling time	MNPCE (% ± S.D.)	
	Male	Female
24 h	1.42±0.06	0.80±0.15
30 h	2.47±0.31	1.53±0.07
36 h	1.63±0.23	1.17±0.04

tional University. Three mice were randomly assigned to each experimental group and given pellet (Samyang Feed Production Co., Korea), which is composed of crude protein 22.1%, crude fat 3.5%, calcium 0.6%, phosphorus 0.4%, crude cellulose 5.0% and crude ash 8.0%, and tap water was supplied *ad libitum*. Experimental mice were adapted with alternating 12 h periods of light/darkness cycle and temperature was controlled with automatic thermostat as 20 ± 2 °C.

Chemicals and treatments

Ascorbic acid was supplied by Hayashi Pure Chemical Industries, Ltd. α-Tocopherol, glutathione (GSH) and benzo(a)pyrene [B(a)P] were obtained from Sigma Chemical Co. Ascorbic acid and GSH were dissolved in distilled water, B(a)P used as carcinogen was diluted with dimethylsulfoxide (DMSO), and α-tocopherol was dissolved in corn oil.

For the time-response and sex difference studies, DEPE was administered at 300 mg/kg body weight by intraperitoneal injection. The bone marrow cells were sampled at 24, 30 or 36h after administration of DEPE. For the dose-response study, DEPE was injected at 100, 200 or 300 mg/kg, respectively. In order to find out the suppressive effect of ascorbic acid, α-tocopherol and GSH toward DEPE-induced micronuclei, each compound was administered at 1, 10 or 100 mg/kg/day for 5 consecutive days in each group of mice. DEPE was administered intraperitoneally once to mice at 200 mg/kg with the 5th administration of test compounds. Except for time-response and sex difference studies, animals were sacrificed 30h after DEPE injection by cervical dislocation. The micronucleus assay was performed according to the method described by Shimid.[17]

Preparation and analysis of slides for micronucleus

The slide were coded and stained with Giemsa solution. Over 1000 polychromatic erythrocytes (PCEs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs). The frequencies of DEPE-induced micronuclei in mice with or without pretreatement of ascorbic acid, α-tocopherol and GSH were compared statistically by Student's t test.

Table II. The frequency of MNPCE induced by DEPE in mouse bone marrow PCE of ddY mice

Dose (mg/kg)	Number of mice tested	MNPCE % individual value			% ± S.D.
Control	3	0.00	0.29	0.38	0.22±0.16
100	3	0.77	0.56	0.59	0.64±0.09*
200	3	1.48	1.32	1.27	1.36±0.09**
300	3	2.57	2.05	2.79	2.47±0.31*

* Significant at $p < 0.05$, compared to controls.
** Significant at $p < 0.01$, compared to controls.

RESULTS

The results of time-response and sex difference in the formation of micronuclei are shown in Table I. At 24, 30 and 36h after DEPE treatment, the frequencies of MNPCE in male mice were 1.42 ± 0.06, 2.47 ± 0.31 and 1.63 ± 0.23%, respectively. The maximal frequency of MNPCE occured at 30h and then decreased. This tendency of DEPE-induced micronuclei was same in female mice. Results in the male and female mice showed that male mice were more sensitive than female mice to DEPE in the mouse bone marrow micronucleus test. MNPCE frequencies, when DEPE at the doses of 100, 200, 300 mg/kg was administered, were found to be 0.64 ± 0.09, 1.36 ± 0.09 and 2.47 ± 0.31, respectively (Table II). With increasing dose of DEPE, the MNPCE frequencies were also increase. At the doses below 300 mg/kg, a linear regression line of $Y = 0.34 + 0.00915X$ ($r = 0.993$) was obtained. In order to find out the effects on the formation of MNPCE by ascorbic acid, α-tocopherol and GSH, its frequencies were compared with B(a)P-induced micronuclei. The results are shown in Table III.

At the dose of 150 mg/kg, B(a)P-induced micronuclei was found to be 1.43 ± 0.03%. Ascorbic acid, α-tocopherol and GSH itself did not induce any MNPCE. On the other hand, DEPE-induced micronuclei (200 mg/kg) was found as 1.36 ± 0.09% (Table II). Such a higher MNPCE frequency of DEPE suggested that DEPE might contain potent mutagens such as nitropyrene as discussed by

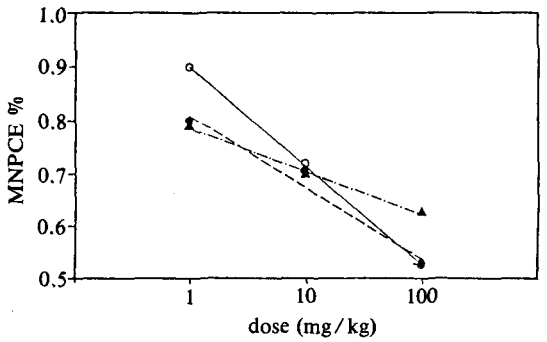

Fig. 1. Dose-dependent suppression of DEPE-induced MNPCEs in ddY male mice by pre-treatment with ascorbic acid, α-tocopherol or GSH.

Test compounds were given orally at the indicated doses for 5 consecutive days, DEPE was administered intraperitoneally once to mice at 200 mg/kg with the 5th administration of test compounds. ○ – ○ Ascorbic acid, ●---● α-tocopherol, ▲-·-·-▲ GSH.

Pitt *et al.*[18]

As shown in Fig. 1, the suppressive effects of pre-treatment with ascorbic acid, α-tocopherol or GSH were investigated at different doses. Each compound was given orally at 1,10 or 100 mg/kg for 5 consecutive days, and the dose of DEPE was fixed at 200 mg/kg. Corresponding to the test compounds doses, the MNPCE frequencies were shown as 0.90 ± 0.09%, 0.72 ± 0.05% and 0.53 ± 0.17%, respectively, in the case of ascorbic acid, 0.80 ± 0.08, 0.70 ± 0.08 and 0.53 ± 0.17%, respectively, in the case of α-tocopherol and 0.79 ± 0.09, 0.70 ± 0.13 and 0.63 ± 0.13%, respectively, in the case of GSH. All the tested compounds significantly reduced MNPCE frequencies in DEPE-induced micronuclei ($p < 0.01$). These results also showed that there were dose-dependent relationships between the dose of ascorbic acid, α-tocopherol or GSH, and the suppressive effect of MNPCE formation of DEPE.

DISCUSSION

The potential carcinogenicity of diesel emissions has been an important subjects in the field of environmental toxicology. Several *in vitro* studies using the Ames test have suggested that DEPE has a strong mutagenic effects.[19-21] The nitro-PAHs have been identified in diesel emission particle extracts and contributed to the observed mutagenic activity in the *Salmonella typhimurium* bacterial assay. And *in vitro* mammalian cell studies of human lymphocyte and CHO cell sister chromatid exchanges demonstrated that DEPE has a strong clastogenic effect.[22,23] This was supported by the finding of Pereira *et al.*[24-26] using the mouse and chinese hamster micronucleus test. In our present work, we also found that the frequency of MNPCE was significantly increased by DEPE in the mouse bone marrow micronucleus test. When one of the three tested compounds was administered in mouse, MNPCE frequencies were not increased, indicating that the tested compounds itself do not play any role to MNPCE formation. However, the pre-treatment of the tested compounds suppressed the frequencies significantly. Besides their general biological importance of some vitamins, in particular, ascorbic acid and α-tocopherol, have shown effective protections against carcinogenic activity of ionizing radiations and chemical agents.[27-29] Comparable anticlastogenic effects of both vitamins on the chromosome

Table III. The frequency of MNPCE induced by ascorbic acid, α-tocopherol, GSH and benzo(a)pyrene into male ddY mice

Compound	Dose (mg/kg)	Number of mice tested	MNPCE % Individual value			% ± S.D.
Control		3	0.00	0.29	0.38	0.22 ± 0.16
DMSO	0.1 ml[a, b]	3	0.49	0.60	0.50	0.53 ± 0.05
Benzo(a)pyrene	150[b]	3	1.45	1.39	1.45	1.43 ± 0.03**
Ascorbic acid, 5 days	100[c]	3	0.30	0.20	0.20	0.23 ± 0.05
α- Tocopherol, 5 days	100[c]	3	0.30	0.30	0.30	0.30 ± 0.00
GSH, 5 days	100[c]	3	0.40	0.39	0.30	0.36 ± 0.04

[a] Dimethylsulfoxide (0.1 ml/mouse)
[b] Intraperitoneal injection
[c] Oral administration
** Significant at $p < 0.01$, compared to DMSO

aberration of carcinogens were reported from human lymphocyte culture by Shamberger *et al.*[30,31] The antimutagenic action of α-tocopherol on the effect of chemical mutagens and carcinogens has been reported from bacteria and mammalian cells.[32,33] The administration of GSH significantly suppressed the frequencies of MNPCEs in the DEPE-induced micronuclei. According to Ito, *et al.*[15] glutathion-s-transferase and glutathione in liver were reported to play important roles in the suppression of dimethyl benzo(a)anthracene induced chromosome aberration by Sudan III. Although its role and mechanism in the prevention of human cancer has not been clearly established, ascorbic acid, α-tocopherol and GSH might be of importance as a protective agent against the genotoxic damages by the chronic exposure of environmental mutagens and/or carcinogens such as diesel emission particles.[34,35] The mechanism of suppressive effect in DEPE-induced micronuclei has not been elucidated yet and there may be different mechanisms. In any case, further studies are required to explain the mechanisms.

In conclusion, induction of micronuclei by DEPE in mouse bone marrow cell was suppressed by pre-treatment with ascorbic acid, α-tocopherol or GSH.

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