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Inhibition of 1,2-Dimethylhydrazine-induced Oxidative DNA Damage by Green Tea Extract in Rat

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Following subcutaneous injection of 1,2-dimethylhydrazine (DMH), which is carcinogenic to rat colon and liver, to Sprague-Dawley rats, a significant increase of 8-hydroxydeoxyguanosine (8-OHdG) was observed in the DNA of colonic mucosa and liver. The 8-OHdG formation reached the maximal level at about 24 h after the DMH injection. On the other hand, no increase of 8-OHdG was observed in the DNA of the kidney. Drinking green tea extract (GTE) for ten days prior to the DMH injection significantly inhibited the formation of 8-OHdG in the colon. These findings demonstrate that DMH causes oxidative damage to the DNA of its target organ, and that GTE protects colonic mucosa from this oxidative damage.

Key words: 8-Hydroxydeoxyguanosine — Green tea — 1,2-Dimethylhydrazine — Colon cancer

Carcinoma of the colon is one of the most common cancers in the developed countries and its prevention is of great interest throughout the world. 1,2-Dimethylhydrazine (DMH) is widely used for experimental studies of colon carcinogenesis in rodents. The main effect of DMH is thought to be a DNA-alkylating action, but involvement of O_2^- in DMH carcinogenesis has also been suspected.[1] 8-Hydroxydeoxyguanosine (8-OHdG) is one of the products of DNA damage by oxygen radicals[2] and its formation in DNA may cause mutagenesis and carcinogenesis as a result of misreading of bases.[3]

Tea has been one of the most commonly consumed beverages since ancient times. There are two common forms, black tea and green tea, depending upon the process of manufacture. Green tea is consumed primarily in China, Japan, and a few countries in North Africa and the Middle East,[4] and it is rich in polyphenolic compounds known as catechins. Several recent reports suggest that it has a suppressive effect on cancer. A case-control study in northern Kyushu indicated a decreased risk of gastric cancer among those with a high consumption of green tea.[5] Many studies on experimental carcinogenesis in animal models have found antitumor effect of polyphenols in green tea.[6-11] We have also reported that green tea extract has a chemopreventive action against azoxymethane-induced colon cancer in rats[12] and that (−)-epigallocatechin gallate (EGCG), the main constituent of Japanese green tea, inhibits N-ethyl-N′-nitro-N-nitrosoguanidine-induced duodenal cancer in mice,[13] though the mechanism of its action is not clear.

We have examined the oxidative action of DMH against DNA *in vivo* by quantifying the formation of 8-OHdG in colonic mucosal DNA, and we evaluated the influence of green tea extract on 8-OHdG formation in order to test the hypothesis that the antitumor effect of green tea involves a protective action against oxidative damage.

MATERIALS AND METHODS

Chemicals and standards 1,2-Dimethylhydrazine dihydrochloride (DMH-2HCl), HPLC-grade methanol, reagent-grade deoxyguanosine (dG), and sodium dodecyl sulfate were purchased from Nacalai Tesque, Inc., Kyoto. 8-OHdG was purchased from Wako Pure Chemical Industries, Ltd., Osaka. Ribonuclease T1 was obtained from Worthington Biochemicals, Freehold, NJ. Ribonuclease A (Type II-A) and alkaline phosphatase (Type III) were purchased from Sigma Chemical Co., St. Louis, MO. Nuclease P1 was obtained from Seikagaku Kogyo, Tokyo. Green tea extract (GTE) was supplied through the courtesy of Taiyo Kagaku Co., Ltd., Yokkaichi, as "Sunphenon." It is composed mainly of polyphenolic compounds: (+)-catechin (3.5%), (−)-epicatechin (7.0%), (+)-gallocatechin (14.8%), (−)-epigallocatechin (15.0%), (−)-epicatechin gallate (4.6%), (−)-gallocatechin gallate (11.6%) and EGCG (18.0%). The residue of GTE includes caffeine, sugars, amino acids and water. DMH was prepared as a 2% solution in distilled water containing 0.1% EDTA, and was adjusted to pH 6.5 with sodium bicarbonate. Saline was used in the control group.

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Animals Five-week-old male Sprague-Dawley rats were purchased from Charles River Japan, Inc., Atsugi, and were housed in metal cages in an air-conditioned (25°C) room with a 12-h dark/12-h light cycle. All rats were given standard diet chow MF from Oriental Yeast, Co., Tokyo. Prior to the experiments, all rats were allowed a one-week acclimation period.

Preparations of samples The first experiment was designed to study how the level of 8-OHdG would change following DMH injection. Rats were divided into two groups, the DMH group and the control group. DMH-treated rats were killed by cervical dislocation at 12, 24, 48, and 96 h after the subcutaneous injection of DMH. The dose of DMH was 25 mg/kg body weight. Control animals were similarly killed after the subcutaneous injection of saline. Each group consisted of eight rats. Laparotomy was performed immediately and the total colon excluding the cecum was extracted. The colon was then washed with ice-cooled saline, incised longitudinally and spread over on ice-cooled glass plate. Colonic mucosa was scraped with a surgical scalpel to remove the muscular layer, frozen in liquid nitrogen, and stored at −80°C until the next procedure.

The second experiment was designed to study the influence of GTE on the DMH-induced formation of 8-OHdG in the colon, kidney and liver of rats. First, the rats were divided into two groups, the GTE group and the water group. The GTE group was supplied with a 0.05% solution of GTE as the sole source of water. The water group was supplied with tap water. After ten days of drinking GTE or tap water, each group was divided randomly into four sub-groups, according to the dose of DMH injected, that is, 0, 25, 50, and 100 mg/kg in the saline alone group, DMH(25) group, DMH(50) group, and DMH(100) group respectively. Each sub-group consisted of eight rats. At 24 h after the injection, all the rats were killed, and the colonic mucosa, left kidney, and liver were collected. Colonic mucosa was prepared as described above. The left kidney and liver were cut into pieces of approximately 1 g, frozen immediately with liquid nitrogen, and stored at −80°C.

DNA isolation The DNA was isolated according to Marmur's method[14] with some modification. That is, samples were lysed first with 2% sodium dodecyl sulfate at 37°C for 30 min, and all procedures were carried out in the dark under argon gas.[15] RNA was removed by digestion with ribonuclease II-A (final concentration, 45 unit/ml) and ribonuclease T1 (final concentration, 45 unit/ml) for 45 min at 37°C between the first and second extractions with chloroform/isoamyl alcohol.[16] After isolation, the DNA was dried and stored under argon gas at −20°C until the next procedure.

DNA digestion DNA samples were dissolved in water at a spectrophotometrically calculated concentration of 300 μg/300 μl, and heat-denatured at 95°C for 3 min. The samples were mixed with 9 μl of 1 *M* sodium acetate buffer (pH 4.8) and 15 units of nuclease P1 and digested by incubation at 37°C for 1 h in the dark. To the samples were then added 54 μl of 1 *M* Tris-HCl buffer (pH 7.2) and 9 units of alkaline phosphatase type III, and incubation was continued at 37°C for 1 h. Finally, the samples were filtered on a Millipore filter (UFC3 LGC 0S, from Nihon Millipore Kogyo K.K., Tokyo). After filtration, the samples were analyzed by HPLC within 5 h.

Determination of 8-OHdG level The HPLC conditions were as follows; columns, Cosmosil 5C18 AR, 4.6 mm × 50 mm (guard column) and 4.6 mm × 250 mm (analytical column) purchased from Nacalai Tesque, Inc., Kyoto; mobile phase, 10 m*M* NaH_2PO_4, 8% methanol, 30 μM EDTA; flow rate, 1.0 ml/min; column temperature, 37°C. The HPLC system (Shimadzu, Kyoto) was as follows: DGU-4A degasser; two LC-10AS pumps; SCL-10A system controller; CTO-10A column oven; SPD-10A UV spectrophotometric detector; ECD-6A electrochemical detector.

The UV spectrophotometric detector was used to detect the concentration of dG at 290 nm, and the electrochemical detector to detect the concentration of 8-OHdG at 700 mV in the oxidation mode. The molar ratio of 8-OHdG to dG was determined from the peak height of 8-OHdG in ECD and the peak area in the case of UV detector. Prior to each series of injections, a standard line was obtained with chromatographic grade samples of 8-OHdG at 50 n*M* and dG at 500 μM.

We expressed the 8-OHdG level as the molar ratio of (8-OHdG/dG) $\times 10^5$ unless otherwise stated. The 8-OHdG level in the standard DNA sample from calf thymus (Boehringer Mannheim, Mannheim), was in the range of 2.50–2.81.

Statistical analysis The results are expressed as the mean ±SD in the text and figures. The significance of differences was tested first by analysis of variance (ANOVA), and if a positive correlation was found, the groups were compared by using the Bonferroni test for multiple comparison. Differences with $P<0.05$ were considered significant unless otherwise noted. All the statistical procedures used a standard software package (SPSS for Windows Release 6; SPSS Inc., Chicago, IL.)[17,18] on a personal computer (IBM PS/V).

RESULTS

Fig. 1 shows the time-related change of 8-OHdG level in the colon at various time points after DMH or saline injection. Two-way ANOVA was carried out first to check the main and interactive effects of time and DMH treatment. Because the null hypothesis that there is no interaction could be rejected, each experimental group

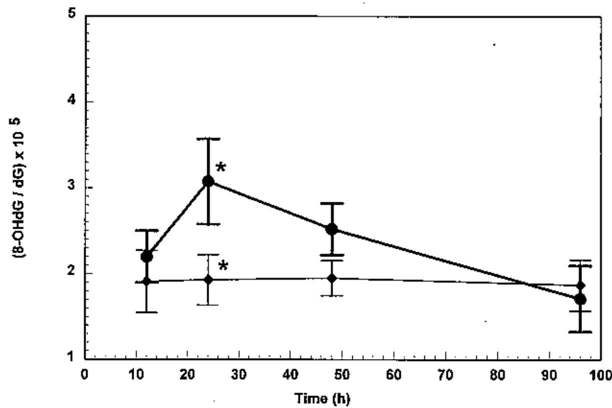

Fig. 1. Time-course of the 8-OHdG level in the colonic mucosal DNA following DMH or saline treatment. The thick line with closed circles (●) represents the DMH group that received 25 mg/kg of DMH, and the thin line with diamonds (◆) represents the control group that received saline injection. Values represent the mean±SD of 8 independent cases. * $P<0.05$.

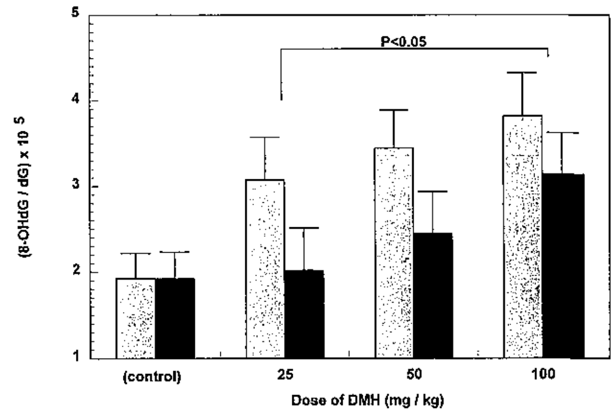

Fig. 2. The level of 8-OHdG in the colon mucosa in the water (light gray columns) and GTE groups (dark gray columns) following injection of various doses of DMH. For reference, the values for the control group (saline injection instead of DMH) are shown on the left. Values represent the mean±SD of 8 independent cases. There is a significant difference between the water and GTE groups ($P<0.01$).

was then rearranged according to the one-way ANOVA model.

The 8-OHdG level in the colonic mucosa in the DMH group at 24 h was 3.08±0.53 and this was significantly higher than that in the control group (1.91±0.39). This result demonstrates that oxidative damage to DNA was generated by the injection of DMH. Fig.1 also shows the peak time to be about 24 h following the DMH injection. At 96 h no significant difference was found between the DMH group (1.71±0.41) and the control group (1.87±0.32). This shows that 8-OHdG is quickly removed from the DNA. In the control group, given saline instead of DMH, no time-related change in the 8-OHdG level was seen.

Fig. 2 shows the relationship of the 8-OHdG level of the colon and the dose of DMH in the groups with and without GTE treatment. As the dose of DMH was increased from 25 mg/kg to 100 mg/kg, the level of 8-OHdG in the colon was elevated. The two-way ANOVA method was used to determine the main and interactive effects of the dose of DMH and GTE treatment. There was no significant interaction, and the three groups that received GTE (that is, DMH(25)-GTE, DMH(50)-GTE, and DMH(100)-GTE) showed significantly lower values of 8-OHdG than the three control groups ($P<0.01$). This shows that GTE inhibited the formation of 8-OHdG at all three concentrations of DMH. There was a significant difference between the DMH(25) and DMH(100) groups. This implies that the formation of 8-OHdG in the colonic mucosa increases in a dose-dependent manner following DMH administration.

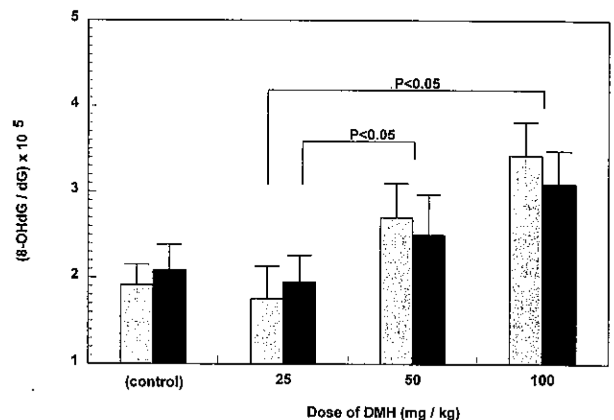

Fig. 3. The level of 8-OHdG in the liver in the water (light gray columns) and GTE groups (dark gray columns) following injection of various doses of DMH. For reference, the values for the control group (saline injection instead of DMH) are shown on the left. Values represent the mean±SD of 8 independent cases. There is no significant difference between the water and GTE groups.

Fig. 3 shows the results obtained for the liver. A similar two-way ANOVA model was employed. For the liver, there was a significant difference between the DMH(25) and DMH(50) groups and between the DMH(25) and DMH(100) groups. This shows that DMH causes oxidative damage to the liver, probably in a dose-dependent manner. It is presumed that GTE also inhibited the formation of 8-OHdG in the liver especially

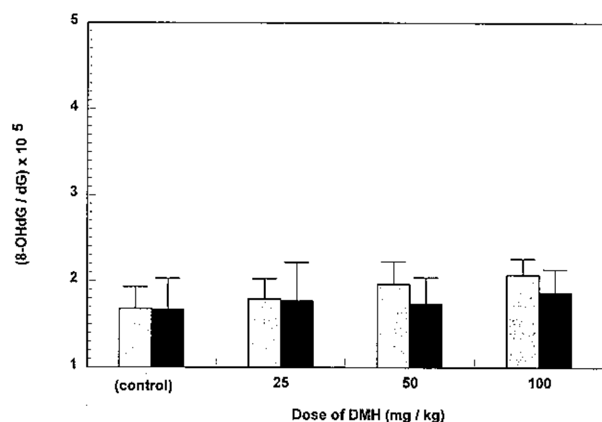

Fig. 4. The level of 8-OHdG in the kidney in the water (light gray columns) and GTE groups (dark gray columns) following injection of various doses of DMH. For reference, the values for the control group (saline injection instead of DMH) are shown on the left. Values represent the mean± SD of 8 independent cases. There is no significant difference between the water and GTE groups.

at the DMH(50) and DMH(100) levels, but the effect of GTE was not statistically significant.

Fig. 4 shows the results for the kidney. There was no significant change in the 8-OHdG level in the kidney following injections of various amounts of DMH. Even at the dose of 100 mg/kg of DMH, the formation of 8-OHdG remained as low as that in the control group.

DISCUSSION

While DMH has been widely used for experimental colon carcinogenesis in rat and mouse models, its mechanism of action is not established. DMH is enzymatically metabolized to methylazoxy methanol, and transformed to methyldiazonium, the ultimate carcinogen, which methylates DNA bases.[19] O^6-Methylguanine is a common adduct of DNA methylation, and Bull *et al.*[20] emphasized that the formation of O^6-methylguanine is responsible for the main effect of DMH. Rogers and Pegg, however, reported that there was less O^6-methylguanine formation in the colon than in the liver of DMH-treated rats.[21] As the target organ of DMH is mainly the colon, not the liver, there may be another factor that plays a greater role in the colon carcinogenesis of DMH. Sun *et al.* suggested the involvement of O_2^- in DMH carcinogenesis[1] and, recently, Kawanishi and Yamamoto showed that the hydroxyl free radical (·OH) is generated during the Mn(III)-catalyzed autooxidation of DMH via O_2^-, and that Cu(I)-peroxide complex participates in the DNA damage induced by methylhydrazine plus Cu(II).[22] In our study, the increased formation of 8-OHdG following DMH treatment supports the hypothesis that the oxidative damage plays a role in the mechanism of action of DMH. The level of 8-OHdG was higher in colonic mucosa than in the liver or kidney at the dose of 25 mg/kg, and the level of 8-OHdG in the colon was increased by DMH in a dose-dependent manner. But this study does not show directly whether the formation of 8-OHdG itself is involved in the initiation stage of DMH-induced colon carcinogenesis.

DMH causes not only colon cancer but also hemangiosarcoma in the liver after long-term oral administration,[23] and so weekly injection of 20 mg/kg for several weeks is the preferred method of producing colon carcinogenesis by DMH. In our study, as the liver was taken as a whole tissue, the increased level of 8-OHdG in the liver in the 100 mg/kg group suggests that the non-parenchymal cell DNA in the liver is damaged by a high dose of DMH. However, there remains much room for discussion regarding the cell-susceptibility to DMH. The removal as well as the formation of DNA adducts must be taken into consideration.[24] We did not examine the time-course of the 8-OHdG level in various organs, and further investigation is needed. In summary, at a low dose of DMH, the colonic mucosa was damaged, and at a high dose, the liver as well as the colon was influenced. These findings also strengthen the view that DMH initiates the oxidative damage of DNA, considering the organ-specificity of DMH. Wei and Frenkel reported that the 8-OHdG level was increased by application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), a tumor promoter, to mouse skin.[25] We did not examine the long-term changes in the 8-OHdG level following DMH treatment by continuous weekly injections, which is the preferred administration method for experimental colon carcinogenesis. Therefore, the 8-OHdG level at the promotion stage of DMH-induced carcinogenesis is our next target for investigation.

Antioxidants, such as vitamin E,[26] disulfiram,[27] and selenium,[28] have been reported to have an inhibitory effect on DMH carcinogenesis. We previously showed that green tea extract inhibits colon carcinogenesis induced by azoxymethane,[29] which is a metabolite of DMH. Furthermore, green tea polyphenol fraction was reported to inhibit DMH-induced colorectal tumors in rats.[30] The green tea extract had a scavenging effect on active oxygen radicals[31, 32] and a polyphenol compound from green tea has been reported to have an antioxidative effect.[33] Recently chemopreventive agents, such as EGCG, sarchophytol A, caffeic acid phenethyl ester, and penta-*O*-galloyl-β-D-glucose, have been reported to inhibit TPA-induced H_2O_2 formation and 8-OHdG formation *in vitro*.[34] We found that green tea extract decreased the 8-OHdG level in the colonic mucosa *in vivo*. This directly demonstrates the antioxidative effect of green tea

polyphenols. However, it is not clear that the antioxidative effect is the main factor in the chemopreventive action of GTE. Since the formation of O^6-methylguanine and the enhancement of cell proliferation are known to be involved in DMH-induced colon carcinogenesis in rats, studies on the effects of GTE on these processes will be required. Narisawa and Fukaura recently reported that green tea could be expected to affect not only the promotion phase, but also the initiation phase of N-methyl-N-nitrosourea-induced colon carcinogenesis.[35] Our findings support their conclusion, in that both studies show an inhibitory effect on the initiation stage of colon carcinogenesis.

Since 1981, cancer has been the greatest cause of death in Japan, and interest in cancer chemoprevention has been increasing. Among the known chemopreventive compounds, green tea polyphenols appear to have many advantages. They are cheap, can be produced in large quantity, and are familiar to the public. Although many laboratory studies including ours have demonstrated that green tea inhibits carcinogenesis in several animal models, epidemiological studies are not conclusive. In order to make further progress in this field, collaboration among clinicians, natural product chemists, and epidemiologists is needed.

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REFERENCES

1) Sun, Y., Li, Y. and Oberley, L. W. Superoxide dismutase activity during dimethylhydrazine colon carcinogenesis and the effects of cholic acid and indole. *Free Radical Res. Commun.*, **4**, 299–309 (1988).
2) Kasai, H., Nishimura, S., Kurokawa, Y. and Hayashi, Y. Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. *Carcinogenesis*, **8**, 1959–1961 (1987).
3) Kuchino, Y., Mori, F., Kasai, H., Inoue, H., Iwai, S., Miura, K., Ohtsuka, E. and Nishimura, S. Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature*, **327**, 77–79 (1987).
4) Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.*, **21**, 334–350 (1992).
5) Kono, S., Ikeda, M., Tokudome, S. and Karatsune, M. A case-control study of gastric cancer and diet in Northern Kyushu. *Jpn. J. Cancer Res.*, **79**, 1067–1074 (1988).
6) Yoshizawa, S., Horiuchi, T., Fujiki, H., Yoshida, T., Okuda, T. and Sugimura, T. Antitumor promoting activity of (−)-epigallocatechin gallate, the main constituent of "tannin" in green tea. *Phytother. Res.*, **1**, 44–47 (1987).
7) Wang, Z. Y., Khan, W. A., Bickers, D. R. and Mukhtar, H. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis*, **10**, 411–415 (1989).
8) Han, C. and Xu, Y. The effect of Chinese tea on occurrence of esophageal tumor induced by *N*-nitrosomethylbenzylamine in rats. *Biomed. Environ. Sci.*, **3**, 35–42 (1990).
9) Wang, Z. Y., Huang, M. T., Ferraro, T., Wong, C. Q., Lou, Y. R., Reuhl, K., Iatropoulos, M., Yang, C. S. and Conney, A. H. Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-*O*-tetradecanoylphorbol-13-acetate in the skin of SKH-1 mice. *Cancer Res.*, **52**, 1162–1170 (1992).
10) Katiyar, S. K., Agarwal, R., Wood, G. S. and Mukhtar, H. Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz[a]anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. *Cancer Res.*, **52**, 6890–6897 (1992).
11) Fujiki, H., Yoshizawa, S., Horiuchi, T., Suganuma, M., Yatsunami, J., Nishiwaki, S., Okabe, S., Nishiwaki, R., Okuda, T. and Sugimura, T. Anticarcinogenic effects of (−)-epigallocatechin gallate. *Prev. Med.*, **21**, 503–509 (1992).
12) Yamane, T., Hagiwara, N., Tateishi, M., Akachi, S., Kim, M., Okuzumi, J., Kitao, Y., Inagake, M., Kuwata, K. and Takahashi, T. Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. *Jpn. J. Cancer Res.*, **82**, 1336–1339 (1991).
13) Fujita, Y., Yamane, T., Tanaka, M., Kuwata, K., Okuzumi, J., Takahashi, T., Fujiki, H. and Okuda, T. Inhibitory effect of (−)-epigallocatechin gallate on carcinogenesis with N-ethyl-N′-nitro-N-nitrosoguanidine in mouse duodenum. *Jpn. J. Cancer Res.*, **80**, 503–505 (1989).
14) Marmur, J. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.*, **3**, 208–218 (1961).
15) Kasai, H., Crain, P. F., Kuchino, Y., Nishimura, S., Ootsuyama, A. and Tanooka, H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing

oxygen radicals and evidence for its repair. *Carcinogenesis,* **7,** 1849–1851 (1986).

16) Fiala, E. S., Conaway, C. C. and Mathis, J. E. Oxidative DNA and RNA damage in the livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane. *Cancer Res.,* **49,** 5518–5522 (1989).
17) Norusis, M. J. and SPSS Inc. "SPSS for Windows: Base System User's Guide, Release 6.0" (1993). SPSS Inc., Chicago.
18) Norusis, M. J. and SPSS Inc. "SPSS for Windows: Advanced Statistics, Release 6.0" (1993). SPSS Inc., Chicago.
19) Fiala, E. S. Investigations into the metabolism and mode of action of the colon carcinogens 1, 2-dimethylhydrazine and azoxymethane. *Cancer,* **40,** 2436–2445 (1977).
20) Bull, A. W., Burd, A. D. and Nigro, N. D. Effect of inhibitors of tumorigenesis on the formation of O^6-methylguanine in the colon of 1,2-dimethylhydrazine-treated rats. *Cancer Res.,* **41,** 4938–4941 (1981).
21) Rogers, K. J. and Pegg, A. E. Formation of O^6-methylguanine by alkylation of rat liver, colon and kidney DNA following administration of 1,2-dimethylhydrazine. *Cancer Res.,* **37,** 4082–4087 (1977).
22) Kawanishi, S. and Yamamoto, K. Mechanism of site-specific DNA damage induced by methylhydrazines in the presence of copper(II) or manganese(III). *Biochemistry,* **30,** 3069–3075 (1991).
23) Bedell, M. A., Lewis, J. G., Billings, K. C. and Swenberg, J. A. Cell specificity in hepatocarcinogenesis: preferential accumulation of O^6-methylguanine in target cell DNA during continuous exposure of rats to 1,2-dimethylhydrazine. *Cancer Res.,* **42,** 3079–3083 (1982).
24) Herron, D. C. and Shank, R. C. *In vivo* kinetics of O^6-methylguanine and 7-methylguanine formation and persistence in DNA of rats treated with symmetrical dimethylhydrazine. *Cancer Res.,* **41,** 3967–3972 (1981).
25) Wei, H. and Frenkel, K. *In vivo* formation of oxidized DNA bases in tumor promoter-treated mouse skin. *Cancer Res.,* **51,** 4443–4449 (1991).
26) Cook, M. G. and McNamara, P. Effect of dietary vitamin E on dimethylhydrazine-induced colonic tumors in mice. *Cancer Res.,* **40,** 1329–1331 (1980).
27) Wattenberg, L. W. Inhibition of dimethylhydrazine-induced neoplasia of the large intestine by disulfiram. *J. Natl. Cancer Inst.,* **54,** 1005–1006 (1975).
28) Pence, B. C. Dietary selenium and antioxidant status: toxic effects of 1,2-dimethylhydrazine in rats. *J. Nutr.,* **121,** 138–144 (1991).
29) Inagake, M., Yamane, T., Kitao, Y., Kuwata, K., Oya, K., Okuyama, A. and Takahashi, T. Inhibitory effect of green tea extract against azoxymethane-induced colon carcinogenesis in rat. *In* "Recent Advances in Management of Digestive Cancers," ed. T. Takahashi, pp. 474–476 (1993). Springer-Verlag, Tokyo.
30) Kan, H., Onda, M., Tanaka, N., Furukawa, K., Yokoi, K., Takasaki, H., Seya, T., Yokoyama, S., Maruyama, H., Kanazawa, Y., Sasabe, H., Yamashita, K., Sakanaka, S. and Kim, M. Effect of green tea catechin fraction in DMH-induced colorectal tumor in rat. *J. Jpn. Res. Soc. Gastroenterol. Carcinog.,* **6,** 45–48 (1994) (in Japanese).
31) Wang, Z. Y., Cheng, S. J., Zhou, Z. C., Athar, M., Khan, W. A., Bickers, D. R. and Mukhtar, H. Antimutagenicity of green tea polyphenols. *Mutat. Res.,* **223,** 273–285 (1989).
32) Zhao, B., Li, X. J., He, R. G., Cheng, S. J. and Xin, W. J. Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. *Cell Biophys.,* **14,** 175–186 (1989).
33) Osawa, T., Namiki, M. and Kawakishi, S. Role of dietary antioxidants in protection against oxidative damage. *In* "Basic Life Science, Vol. 52, Antimutagenesis and Anticarcinogenesis Mechanisms II," ed. Y. Kuroda, D. M. Shankel and M. D. Waters, pp.139–153 (1990). Plenum Press, New York.
34) Bhimani, R. S., Troll, W., Grunberger, D. and Frenkel, K. Inhibition of oxidative stress in HeLa cells by chemopreventive agents. *Cancer Res.,* **53,** 4528–4533 (1993).
35) Narisawa, T. and Fukaura, Y. A very low dose of green tea polyphenols in drinking water prevents N-methyl-N-nitrosourea-induced colon carcinogenesis in F344 rats. *Jpn. J. Cancer Res.,* **84,** 1007–1009 (1993).