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Effects of Flavonol Derivatives on the Micronuclei Formation by N-methyl-N'-nitro-N-nitrosoguanidine and the Enhancement of Bleomycin-induced Chromosome Aberration by N-methyl-N'-nitro-N-nitrosoguanidine

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Flavonol derivatives were tested for their anticlastogenic effect against induction of micronuclei by N-methyl-N'-nitro-N-nitrosoguanidine(MNNG), and against induction of chromosome aberration by bleomycin or MNNG/bleomycin. For micronucleus assay, each flavonol derivative (0, 0.001, 0.01, 0.1, 1, 10 and 100 mg/kg) was administered orally twice with 24 h interval, together with intraperitoneally administered MNNG (150 mg/kg). The results showed that most flavonol derivatives tested were effective in suppressing the frequencies of micronuclei induced by MNNG. For chromosome aberration assay, each flavonol derivative (0, 0.1, 1, 10, and 100 mg/kg)was administered to mice orally *in vivo*, and then mice were sacrificed and spleen lymphocyte cultures were made. Bleomycin (3 μg/ml) was treated to the mouse spleen lymphocyte cultures at 24 h after con A initiation. There were no marked decrease tendencies in chromosome aberration unless all doses of galangin and some doses of several flavonol derivatives tested. In the another experiment, we have evaluated the effect of flavonol derivatives on the enhancement of bleomycin-induced chromosome aberration by MNNG. Most of flavonol derivatives reduced the incidence of chromosome aberration induced by in vitro treatment of bleomycin followed by *in vivo* treatment of MNNG. Galangin particulary showed a dose-dependent decrease tendency. Other flavonol derivatives showed slightly decrease tendencies although there were no dose-dependent relationships. These results suggest that most of flavonol derivatives may be capable of protecting the inhibition of DNA-repair by MNNG. Our data indicate clearly that flavonol derivatives can suppress MNNG-induced genotoxicity such as an induction of MNPCEs. Therefore, our results could suggest that flavonol derivatives may be useful as a chemopreventive agent of MNNG.

Key words: Flavonoids, Flavonol derivatives, Anticlastogenic effect, Micronucleus assay, Chromosome aberration assay, N-methyl-N'-nitro-N-nitrosoguanidine(MNNG), Bleomycin

INTRODUCTION

Recently it has become more important to evaluate the factor which modify the mutagenic or carcinogenic activities of environmental contaminants. The flavonoids are one of the most common plant metabolites. They are widely distributed in the fruits, vegetables, and beverages in our diet as well as many medicinal plants (Kuhnau, 1976). Because a large amount of flavonoids are consumed by human, these compounds have been intensively tested for their genotoxic and carcinogenic potential(Herman, 1976; Brown, 1980; Elliger *et al.*, 1981; Nagao *et al.*, 1981). These compounds have been reported to have a broad spectrum of biological activities (Cody *et al.*, 1986) including frequently reported mutagenic (MacGregor, 1986) and less frquently reported antimutagenic activities (Mitscher *et al.*, 1985). Wall *et al.* (1988) found that galangin, neobavaisoflavone, and bakuthiol were highly antimutagenic and nontoxic against 2-aminoanthracene. Birt *et al.* (1986) have reported that apigenin and robinetin had strong inhibition in 2-aminoanthracene and were not toxic and not less activity in benzo

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(a)pyrene. Mitscher *et al.*(1985) showed that glabrene was highly antimutagenic and nontoxic against ethylmethane sulfonate-induced mutation.

Until now, most of antimutagenic studies were performed with procaryotic system. Few studies have been conducted with mammalian cell, especially in *in vivo* system. We have previously reported that flavonoid derivatives showed an anticlastogenic effect(Heo *et al.*, 1992). We have documented that 14 flavonoids, including flavone and flavonol derivatives, were tested for their anticlastogenic effects against induction of micronuclei by benzo(a)pyrene in polychromatic erythrocytes of bone-marrow in mice. We found that several flavonoids, mainly flavonol derivatives having 2,3-double bond and 3,5,7-trihydroxyl groups in the flavonoid molecules, showed potent anticlastogenic effects against secondary mutagen, benzo(a)pyrene-induced micronuclei. Therefore, it may be worthy of studying the anticlastogenic effect of the flavonol derivatives against different types of mutagen such as direct mutagen, MNNG and radiomimetic agent, bleomycin. In this study, we have investigated an anticlastogenic effects of flavonol derivatives on the frequency of MNPCEs induced by MNNG in mouse bone-marrow cells or the chromosome aberrations induced by bleomycin or MNNG/bleomycin in mouse spleen lymphocytes.

MATERIALS AND METHODS

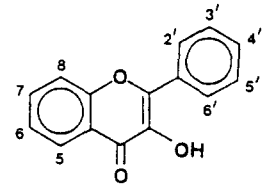
Chemicals

Favonol and kaempferol were obtained from Tokyo Kasei Chemical Company, Tokyo, Japan. Other flavonols were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, USA (Fig. 1). N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, CAS No.70-25-7) was obtained from Fluka Chemical Company, Swizerland. Bleomycin was obtained Dong-A Pharmaceutical Company, Seoul, Korea. The CAS Nos. for the various flavonoids are; fisetin (528-48-3); galangin (548-83-4); kaempferol (520-18-3); morin (480-16-0); quercetin (651-25-3). Flavonol derivatives were dissolved in corn oil. MNNG was dissolved in dimethyl sulfoxide (DMSO). Bleomycin was dissolved in distilled water. In all experiments, the administration volume was 0.1 ml/25 g of body weight orally or intraperitoneally.

Animals

Male ICR and C57BL/6 mice(15-20 g) which were obtained from the Animal Center of the Seoul National University (Seoul, Korea). Mice were maintained in the chambers with laminar air flow at a temperature of 22± 1℃ and 12 hr/12 hr (LD) cycle on the standard diet and water ad libitum.

Mouse bone-marrow micronucleus assay



	5	7	2'	3'	4'	5'
Flavonol	H	H	H	H	H	H
Galangin	OH	OH	H	H	H	H
Fisetin	H	OH	H	OH	OH	H
Morin	OH	OH	OH	H	OH	H
Kaempferol	OH	OH	H	H	OH	H
Qercetin	OH	OH	H	OH	OH	H

Fig. 1. Chemical structure of flavonol derivatives.

In order to know an anticlastogenic activity against MNNG-induced micronuclei in bone-marrow cells of ICR mice, each flavonol derivative (0, 0.001, 0.01, 0.1, 1, 10 and 100 mg/kg) was administered orally twice with 24 h interval. Five mice were randomly assigned to each treatment group. MNNG (150 mg/kg) was administered immediately and intraperitoneally after the dose of flavonol derivatives. Mice were treated with corn oil or DMSO as vehicle controls. The treated mice were sacrificed at 32 h after the treatment with first dose of MNNG by cervical dislocation. The bone-marrow preparations were performed according to Shimid (1975) and 1,000 polychromatic erythrocytes (PCEs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs). And PCEs per 400 red blood cells in each mouse were determined to know bone-marrow effect of chemicals. For the statistical analysis, Student's t-test and Analysis of Variance (ANOVA) test were used to compare the flavonol derivative-treated groups with the untreated group.

Mouse spleen lymphocyte chromosome aberration assay

The procedure for this assay was modified from used by Au, *et al.* (1988). The removal of spleen and the isolation of spleen lymphocytes for cultures were handled aseptically. The spleens from C57BL/6 mice were rinsed in RPMI 1640 culture medium (Gibco No. 380-2400) and were smashed by the sterilized plunger. The cells were spun down and a cell count was made. Cultures were set up with 20×10^6 lymphocytes/ml of complete RPMI 1640 medium. The complete medium contains RPMI 1640 medium, 15% heat inactivated fetal bovine serum (Gibco No. 200-6140), 1% sodium heparin (1,000 unit, Invenex No. 33-10), 1% penicillin-streptomycin (Gibco No. 600-5145), 2% concanavalin A (con A, Sigma No. C-5275), and 0.01% β-mercapto-

ethanol(0.05 M, Sigma No. M-6250). Then the culture tubes were incubated at 37℃ in humidified 5% CO_2 incubator for 42 h. At 6 h after con A initiation, 5-bromo-2'-deoxy uridine (Sigma No.B-5002) was added to each tube.

To evaluate the dose-response relationships of bleomycin, cultures were treated with concentrations of 0, 1, 2, 3, and 5 μg/ml of bleomycin at 24 h after con A initiation. Four cultures per group were performed. For anticlastogenicity test, each flavonol derivative at 0, 0.1, 1, 10 and 100 mg/kg body weight were administered orally twice with 24 h interval. The treated mice were sacrificed at 8 h after treatment with last dose of tested compound by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with 3 μg/ml of bleomycin at 24 h after con A initiation.

In another experiment, the effects of MNNG on bleomycin-induced chromosome aberration in mouse spleen lymphocytes were evaluated. MNNG (0, 25, and 50 mg/kg, body weight) was administered to mice intraperitoneally twice with 24 h interval. Treated mice were sacrificed at 8 h after treatment with last dose of MNNG by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with or without 3 μg/ml of bleomycin at 24 h after con A initiation. In order to evaluate the effects of flavonol derivatives on the enhancement of bleomycin-induced chromosome aberration by MNNG, tested compound (0, 1, 10, and 100 mg/kg, body weight) was administered orally together with treatment of MNNG. Treated mice were sacrificed at 8 h after treatment with last dose of MNNG by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Usually, 2 cultures were made from each mouse. Cultures were treated with 3 μg/ml of bleomycin at 24 h after con A initiation.

Other conditions of culture were same as early mentioned procedure. Cells were harvested and air-dried cytological preparations were made. Slides were prepared and stained with fluorescent-plus-Giemsa technique to score first metaphases. Cells with 46 chromosomes were analyzed and 50 metaphases were scored per culture.

RESULTS

Effect of flavonol derivatives on MNNG-induced micronuclei in mouse bone-marrow cells

In order to establish the proper treatment method and the dose level of MNNG, various concentrations of MNNG were injected intraperitoneally at double treatment with 24 h interval. Mice were sacrificed at 32 h after the first dose of MNNG for micronucleus assay. The frequencies of MNPCEs showed a dose-dependent increase in the double treatment (Data not shown). A double dose of 150 mg/kg was chosen to evaluate an anticlastogeicity of flavonol derivatives.

In the subsequent experiment, flavonol derivatives were orally administered at 0, 0.001, 0.01, 0.1, 1, 10 and 100 mg/kg body weight with a concomitant injection of MNNG. A summary of the data on the frequencies of MNPCEs in animals treated with flavonol derivatives and MNNG simultaneously is shown in Table I. There were statistically significant decreases in the frequency of MNPCEs in mouse bone-marrow cells treated with various concentrations of flavonol derivatives and MNNG simultaneously, even at 0.1 mg/kg body weight. However, there were no decreases less than at 0.01 mg/kg except 0.01 mg/kg of flavonol.

Table V shows the frequency of MNPCEs in bone-marrow cells in mice treated with flavonol derivatives alone. There were no significant increases compared to negative control. From the data presented in Table V, oral administrations of flavonol derivatives alone did'nt show any clastogenicity at 100 mg/kg which was tested with highest dose in this experiment. And there was no suppression in PCE/NCE.

Effects of flavonol derivatives on bleomycin-induced chromosome aberration in mouse spleen lymphocytes

Before we examined an anticlastogenic effect of galangin on bleomycin-induced chromosome aberrations, the dose-response relationship of bleomycin was tested in the mouse spleen lymphocytes. Bleomycin showed a strong clastogenicity with dose-dependent increase at 1 μg/ml-5 μg/ml of medium (Data not shown). Bleomycin-induced chromosome aberration showed mainly gaps and breaks. Cells with exchanges and translocations were observed infrequently. The cells which treated with solvent alone as negative control showed only a few gaps and breaks. We did not include cells with gaps in the class of damaged cells, because gaps are not good indicator of chromosomal damage. The concentration of bleomycin was fixed at 3 μg/ml of medium for anticlastogenicity test.

The incidenckes of chromosome aberration in mouse spleen lymphocyte after *in vivo* treatment with flavonol derivatives are given in Table II. There was no marked decrease tendencies in chromosome aberration unless all doses of galangin and some doses of several flavonol derivatives tested. Especially, galangin reduced dose-dependently the incidence of chromosome aberration induced by MNNG.

Fisetin and morin also showed a decrease at high

Table I. Suppression of MNNG-induced MNPCEs by flavonol derivatives in bone-marrow cells of ICR mice[a]

Flavonols (mg/kg, p.o.)		MNNG (150 mg/kg, i.p.)	MNPCEs/1000cells[d] mean± SE	PCE/NCE[e] mean± SE
—[b]		—[b]	1.19± 0.37	1.10± 0.05
—[c]		+[c]	7.54± 0.50	0.75± 0.03
Fisetin	10^{3}	+	6.68± 0.38[f]	0.59± 0.09
	10^{2}	+	5.86± 0.89[i]	0.44± 0.11
	10^{1}	+	5.92± 1.25[i]	0.52± 0.04
	1	+	3.94± 0.32[h,i]	0.69± 0.02
	10	+	3.91± 0.68[g,i]	0.58± 0.06
	100	+	3.35± 0.74[g,i]	0.70± 0.10
Flavonol	10^{3}	+	8.94± 1.45[i]	0.43± 0.03
	10^{2}	+	4.43± 1.15[f,i]	0.37± 0.02
	10^{1}	+	3.97± 0.56[g,i]	0.57± 0.03
	1	+	4.12± 0.20[h,i]	0.71± 0.09
	10	+	3.64± 0.67[g,i]	0.85± 0.31
	100	+	3.35± 0.67[h,i]	0.67± 0.07
Galangin	10^{3}	+	7.36± 1.71[i]	0.51± 0.04
	10^{2}	+	7.17± 1.45[i]	0.44± 0.02
	10^{1}	+	3.93± 0.75[g,i]	0.66± 0.04
	1	+	3.15± 0.46[h,i]	0.60± 0.06
	10	+	4.75± 1.15[f,i]	0.76± 0.12
	100	+	3.58± 0.51[h,i]	0.82± 0.08
Kaempferol	10^{3}	+	not tested	not tested
	10^{2}	+	6.75± 1.07[i]	0.54± 0.06
	10^{1}	+	3.55± 0.40[h,i]	0.68± 0.04
	1	+	4.16± 0.73[g,i]	0.62± 0.08
	10	+	1.37± 0.25[h,i]	0.58± 0.04
	100	+	3.78± 1.42[f,i]	0.59± 0.02
Morin	10^{3}	+	8.97± 3.03[i]	0.47± 0.03
	10^{2}	+	6.11± 2.42[i]	0.62± 0.14
	10^{1}	+	3.38± 0.92[g,i]	1.07± 0.13
	1	+	3.31± 0.40[h,i]	0.96± 0.11
	10	+	3.32± 0.95[g,i]	0.85± 0.09
	100	+	1.57± 0.49[h,i]	0.70± 0.11
Quercetin	10^{3}	+	9.74± 1.36[i]	0.53± 0.06
	10^{2}	+	8.72± 1.64[i]	0.60± 0.05
	10^{1}	+	4.36± 0.97[f,i]	0.62± 0.05
	1	+	3.01± 0.67[h,i]	0.65± 0.06
	10	+	4.47± 0.85[f,i]	0.77± 0.04
	100	+	2.65± 0.44[f,i]	0.93± 0.07

[a]Flavonol derivatives were administered orally twice with 24 h interval. MNNG (150 mg/kg) was administered immediately and intraperitoneally after the dose of flavonol derivatives. The treated mice were sacrificed at 32 h after the treatment with first dose of MNNG by cervical dislocation. 1,000 polychromatic erythrocytes (PCEs) were scored per mouse to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs).

[b]Mice were treated with corn oil and DMSO as vehicle controls.

[c]Positive control.

[d]Five mice were usually assigned to each treatment group. All data indicates mean SE of at least three mice per group.

[e]PCEs per 400 red blood cells in each mouse were determined.

[f]$p<0.05$, [g]$p<0.01$, [h]$p<0.001$; Student's t-test

[i]$p<0.01$; Analysis of Variation test.

doses, but showed an increase rather than a decrease at low doses. Quercetin was no effective. Flavonol and kaempferol enhanced with increase of administrative dose.

Table II. Effects of bleomycin-induced chromosome aberration by flavonol derivatives in spleen cells of C57BL/6 mice[a]

Flavonols (mg/kg, p.o.)		Bleomycin (3 μg/ml)	No. of metaphase	No. of aberration[d] cdb	cdx	cmb	cme	total	Aberration %	Aberrant %
–[b]		–[b]	200	0	0	0	0	0	0.0	0.0
–[b]		+[b]	200	0	0	0	0	0	0.0	0.0
–[c]		+[c]	200	12	0	11	2	25	12.5	10.0
Fisetin	1	+	200	7	0	13	1	21	10.5	9.0
	10	+	200	12	2	15	2	31	15.5	14.0
	100	+	200	2	2	4	0	8	4.0	3.5
Flavonol	1	+	200	9	1	12	0	22	11.0	10.0
	10	+	200	12	4	10	1	27	13.5	12.5
	100	+	200	12	6	12	0	30	15.0	13.5
Galangin	1	+	200	10	0	14	0	24	12.0	11.0
	10	+	200	6	6	4	0	16	8.0	7.5
	100	+	200	5	2	5	0	12	6.0	5.0
Kaempferol	1	+	200	5	0	11	1	17	8.5	7.0
	10	+	200	17	5	11	3	36	18.0	14.5
	100	+	200	15	16	38	10	79	39.5	31.5
Morin	1	+	200	10	4	29	0	43	21.5	18.5
	10	+	200	11	5	29	1	46	23.0	19.5
	100	+	200	6	8	9	0	23	11.5	9.5
Quercetin	1	+	200	15	2	5	0	22	8.5	8.5
	10	+	200	13	1	3	0	17	8.5	7.5
	100	+	200	12	3	9	1	25	12.5	10.0

[a]Flavonol derivatives were administered orally twice with 24 h interval. The treated mice were sacrificed at 8 h after treatment with last dose of tested compound by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with 3 μg/ml of bleomycin at 24 h after con A initiation. Cells with 46 chromosomes were analyzed and 50 metaphases were scored per culture.
[b]Negative control
[c]Positive control
[d]cdb; chromatid break, cdx; chromatid exchange, cmb; chromosome break, cme; chromosome exchange (translocation and ring).

Table III. Enhancement of chromosome aberration induced by *in vitro* treatment of bleomycin followed by *in vivo* treatment of MNNG[a]

MNNG (50 mg/kg, i.p.)	Bleomycin (3 μg/ml)	No. of metaphase	No. of aberration[c] cdb	cdx	cmb	cme	total	Aberration %	Aberrant %
–[b]	–[b]	200	0	0	0	0	0	0.0	0.0
25	–	200	1	0	0	0	1	0.5	0.5
50	–	200	2	0	0	0	2	1.0	1.0
–	3	200	16	1	8	1	26	13.0	9.0
25	3	200	26	4	9	0	39	19.5	13.5
50	3	200	26	7	11	1	45	22.5	12.5

[a]MNNG was administered to mice intraperitoneally twice with 24 h interval. The treated mice were sacrificed at 8 h after treatment with last dose of MNNG by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with or without 3 μg/ml of bleomycin at 24 h after con A initiation.
[b]Negative control.
[c]cdb; chromatid break, cdx; chromatid exchange, cmb; chromosome break, cme; chromosome exchange (translocation and ring).

In another experiment, Table III shows the incidence of chromosome aberration induced by *in vitro* treatment of bleomycin followed by *in vivo* treatment of MNNG. The chromosome aberrations induced by bleomycin alone was significantly potentiated by the pretreatment of MNNG although MNNG induced no increase of chromosome aberration compared to negative control. MNNG at 50 mg/kg enhanced the frequencies of chromosome aberration about 1.7-fold. For the subsquent study, the treatment of 50 mg/kg,

Table IV. Effects of flavonol derivatives on the enhanncement bleomycin-induced chromosome aberration by MNNG in spleen cells of C57BL/6 mice[a]

Flavonols (mg/kg, p.o.)		MNNG (50 mg/kg, i.p.)	Bleomycin (3 μg/ml)	No. of aberration[d] cdb	cdx	cmb	cme	total	Aberration %	Aberrant %
—[b]		—[b]	—[b]	0	0	0	0	0	0.0	0.0
—[c]		+[c]	+[c]	26	7	11	1	45	22.5	12.5
Fisetin	1	+	+	6	3	0	0	9	23.1	12.8
	10	+	+	6	6	3	2	17	8.5	6.3
	100	+	+	13	1	4	0	18	9.0	5.6
Flavonol	1	+	+	6	3	12	0	21	10.5	8.5
	10	+	+	8	1	8	1	18	9.0	8.5
	100	+	+	6	2	15	0	23	11.5	10.3
Galangin	1	+	+	7	2	3	0	12	6.0	5.0
	10	+	+	1	0	0	0	1	2.0	2.0
	100	+	+	1	0	2	0	3	1.5	1.5
Kaempferol	1	+	+	12	8	24	2	46	23.0	16.5
	10	+	+	18	4	10	2	34	17.0	14.0
	100	+	+	16	4	18	4	42	21.0	13.0
Morin	1	+	+	3	6	20	8	37	17.5	17.5
	10	+	+	18	10	22	1	51	25.5	19.5
	100	+	+	10	0	27	3	40	25.8	16.8
Quercetin	1	+	+	18	4	10	7	39	19.5	15.0
	10	+	+	8	5	21	5	39	19.5	15.5
	100	+	+	7	3	10	1	21	10.5	8.5

aFlavonol derivatives were administered orally together with treatment of MNNG. The treated mice were sacrificed at 8 h after treatment with last dose of MNNG by cervical dislocation. Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with 3 μg/ml of bleomycin at 24 h after con A initiation.

[b]Negative control, [c]Positive control

[d]cdb; chromatid break, cdx; chromatid exchange, cmb; chromosome break, cme; chromosome exchange (translocation and ring).

Table V. Clastogenic effects of flavonol derivatives in micronucleus assay and chromosome aberration assay[a]

Flavonols	Dose (mg/kg,p.o.)	MNPCEs/1000cells mean± SE	PCE/NCE mean± SE	Aberration %	Aberrant %
negative control		1.19± 0.37	1.10± 0.05	0.0	0.0
Fisetin	100	0.39± 0.24	1.13± 0.05	1.0	1.0
Flavonol	100	0.78± 0.36	0.98± 0.13	0.0	0.0
Galangin	100	2.78± 0.49	1.27± 0.13	3.0	3.0
Kaempferol	100	1.37± 0.51	1.07± 0.09	1.0	1.0
Morin	100	1.56± 0.24	0.93± 0.06	2.0	2.0
Quercetin	100	2.65± 0.44	0.93± 0.07	0.5	0.5

[a]Flavonol derivatives were administered orally twice with 24 h interval. MNNG (150 mg/kg) was administered immediately and intraperitoneally after the dose of flavonol derivatives. Mice were treated with corn oil or DMSO as vehicle controls. The treated mice were sacrificed at 32 h after the treatment with first dose of MNNG by cervical dislocation. For micronucleus assay, 1,000 polychromatic erythrocytes (PCEs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MNPCEs). For chromosome aberration assay, Spleen lymphocyte cultures were performed immediately. Each spleen was separately set up from 4 mice per group. Cultures were treated with 3 μg/ml of bleomycin at 24 h after con A initiation. Cells with 46 chromosomes were analyzed and 50 metaphases were scored per culture.

MMNG was chosen to evaluate the effects of flavonol derivatives on enhancement of bleomycin-induced chromosome aberration by MNNG in mouse spleen lymphocytes.

Table IV showed that most of flavonol derivatives reduced the incidence of chromosome aberration induced by MNNG/bleomycin. Galangin particulary showed a potent anticlastogenic effect with dose-dependent tendency. Other flavonol derivatives showed slightly decrease although there were no dose-dependent relationships. Table V shows the incidence of chromosome aberration in spleen lymphocytes in mice

treated with flavonol derivative alone. There were no increase in the chromosome aberration compared to negative control.

DISCUSSION

As we have shown in Table I, MNPCE frequencies in bone-marrow cells of mice treated with MNNG were suppressed by most of flavonol derivatives tested. This phenomenon was similar to results in our previous studies. 14 flavonoids including flavonol derivatives showed to suppress the frequency of MNPCEs by benzo(a)pyrene in bone-marrow cells of mice. The data suggested that flavonol derivatives, 2,3-double bond and 3,5,7-trihydroxyl groups in the flavonoid molecules may be essential to produce an anticlastogenic effect against benzo(a)pyrene. Galangin, one of active flavonol derivatives, against benzo(a)pyrene-induced MNPCEs in mice might be due to the direct inhibition of arylhydrocarbon hydroxylase and the blocking of benzo(a)pyrene-DNA adduct formation (Kim *et al.*, 1991).

We have not yet determined the mechanism which enables flavonol derivatives to suppress the frequency of MNPCEs induce by MNNG in the present study. The genotoxic action of MNNG is thought to involve its decomposition to short-lived, high reactive electrophiles, of which the alkonium ion is probably the ultimate mutagen (Magee *et al.*, 1976). Electrophillic attack on nucleophillic sites of DNA bases, some of which result in base-substition mutation (Loveless, 1969; Nicoll *et al.*, 1975). It is therefore possible that flavonol derivatives, galangin may have a scavenging effect to react with alkonium ion or protect to bind between DNA and alkonium ions.

Some investigator have reported that the mutagenicity of MNNG is inhibited by several classes of chemical including thiols, metals, vitamins, synthetic phenolics and others. The overall activity profile of compounds antimutagenic to MNNG has been subdivided according to the mechanistic classifications of Ramel *et al.* (1986). N-acetyl cysteine (Wilpart *et al.*, 1985), p-amino benzoic acid (Gichner *et al.*, 1987), ascorbic acid (Guttenplan, 1978), cysteamine (Rosin and Stich, 1979), propyl gallate (Rosin and Stich,1979), gallic acid (Stich and Rosin, 1984) and sod. selenite (Whiting *et al.*, 1981) were directly active in the extracellular environment. In the intracellular environment, several classes of antimutagenic agents were distinguished: blocking agents, radical scavengers, agents that affect DNA repair. Among the blocking agents, the phenolics caffeic and chlorogenic acid inhibited a mutagenicity in Samonellar typhimurium (Chan *et al.*, 1986). Sod. selenite inhibited the induction of chromosomal damages by MNNG in CHO cells in vitro (Whiting *et al.*, 1981). But, the radical scavenger and natural phenolic compound, tocopherol was inactive against MNNG in strain TA 1535 (Rosin and Stich, 1979). Cobaltous chloride (Kada and Kanematsu, 1978) and sod.arsenite (Nunoshiba and Nishioka, 1987) were thought to enhance error-free DNA repair. Therefore, there are possibillities that the anticlastogenic effects observed in *in vivo* miccronuleus assay might be due to the scavenging effect to react with alkonium ion or blocking to bind between with DNA and alkonium ion although other possible mechanism such as an enhancement of DNA-repair system could not be excluded.

On the other hand, our results indicate that most of flavonol derivatives tested except galangin were ineffective in suppressing chromosome aberration induced by bleomycin. Bleomycin is a radiomimetic agent. This drug produce both chromosome- and chromatid-type aberration in mammalian cell culture system. Bleomycin acts on chromosomes in an S-independent fashion. The types of aberrations produced by treatment in various phases of the cell cycle are both double and single strand breaks by bleomycin (Vig and Lewis, 1978; Povirk, 1983; Povirk and Finly Austin, 1991). Gebhart (1978) has reported that β-aminoethyl-isothiouronium and homocystein thiolactone greatly reduced the numbers of aberration induced in human lymphocytes treated simultaneously with these agents and bleomycin as compared to cultures treated with bleomycin alone. Pohl and Reidy (1989) found a reduction in bleomycin-induced aberrations in the cultured lymphocytes of individuals after receiving dietary supplementation with 1 g of vitamin C per day for two and four weeks. We may therefore consider that galangin only among flavonol derivatives tested protect DNA damages or activate DNA repair processes due to bleomycin in mouse spleen lymphocyte cultures.

In the next experiment, the challenge assay was performed to be based on our hypothesis that chemicals that can bind to cellular macromolecules are able to interfere with normal cellular fuctions such as DNA repair process and cause inhibitions in DNA repair. The assay was conducted by exposing mice *in vivo* first to the target chemical and then to bleomycin *in vitro* spleen lymphocytes. Mouse spleen cells were therefore challenged to repair the bleomycin-induced DNA lesion in the absence of MNNG. If pretreatment with MNNG cause inhibitions in the repair process, chromosome aberrations induced by bleomycin would potentiated. As shown in the Table III , MNNG potentiated significant increase of chromosome aberrations. The observed phenomenon also showed that MNNG at 25 mg/kg and 50 mg/kg was not clastogenic by itself. It was well established that MNNG forms methylated adducts at various sites of DNA strand, such as N7-, O6-methylated guanine. Therefore, these adducts may interfere the repair of DNA damage indu-

ced by bleomycin.

To evaluate further the effect of flavonol derivatives on the enhancement of bleomycin-induced chromosome aberration by MNNG, flavonol derivative was administered orally together with MNNG injection. The treated mice were sacrificed at 8 h after treatment with last dose of MNNG by cervical dislocation, and then spleen lymphocyte cultures were performed immediately. As shown in Table IV, most of flavonol derivatives reduced the incidence of chromosome aberration induced by MNNG/bleomycin. Galangin particulary showed a dose-dependent decrease tendency. Other flavonol derivatives showed slightly decrease tendencies although there were no dose-dependent relationships. This result suggests that most of flavonol derivatives may be capable of protecting the inhibition of DNA-repair by MNNG. Also, these data indicated clearly that flavonol derivatives can suppress MNNG-induced genotoxicity including the induction of MNPCEs although they had no effect against bleomycin-induced chromosome aberration except galangin.

Table V show the induction of MNPCEs in mouse micronucleus assay and chromosome aberration in mouse spleen lymphocyte culture treated with flavonol derivatives alone. There were no increase of induction in MNPCEs and chromosome aberration compared to negative control. Therefore , flavonol derivatives tested are not clastogenic themselves.

In conclusion, we have reported that flavonol derivatives tested showed suppressive activities against MNNG-induced MNPCEs in mouse bone-marrow cells, and against enhancement of bleomycin-induced chromosome aberration by MNNG in mouse spleen lymphocyte. Our results could suggest that flavonol derivatives may be useful as a chemopreventive agent of MNNG. Therefore, these compounds may be capable of protecting carcinogensis. And more intensive research is required to find out the action mechanisms of flavonol derivatives against variable genotoxic agents.

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