응용약물학회지, The Journal of Applied Pharmacology 2, 183-187(1993)

Anticlastogenic Effects of Galangin against *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine-induced Micronuclei in Bone-marrow Cells of C57BL/6 Mice

Su Jun LEE[1], Chang Ho KWON[2], Kyeong Ho KIM[3], Dong Hun SOHN[2], Moon Young HEO[3]* and William W. AU[4]

[1]*College of Natural Sciences, Kangweon National University, Chuncheon 200-701, Korea*
[2]*College of Pharmacy, Chungang University, Seoul 156-756, Korea*
[3]*College of Pharmacy, Kangweon National University, Chuncheon 200-701, Korea*
[4]*Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston 77555-10100, U.S.A.*

(Received August 17, 1993; accepted November 2, 1993)

Abstract—The anticlastogenic effect of galangin, flavonoid derivative, was studied *in vivo* micronucleus test using C57BL/6 mice. The frequencies of micronuclei induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in bone-marrow cells of C57BL/6 mice were significantly decreased by the simultaneous treatment or multiple pre-treatment of galangin.

When galangin was orally administered at 0, 0.1, 1.0, or 10.0 mg/kg twice with 24 hr interval, together with intraperitoneally administered MNNG, there were suppressive effects in the tested doses. The most marked suppressive effect was observed in the treatment group of 1.0 mg/kg (64.5%), not in the treatment group of 10.0 mg/kg (36.3%). When galangin was multiply administered at 1/7 or 1 mg/kg for 7 days respectively, galangin showed higher suppressive effect in the treatment group of 1/7 mg/kg (23.5%) rather than in the treatment group of 1 mg/kg (13.5%). In another experiment, galangin was administered at 0.001, 0.01 or 0.1 mg/kg for 1 month respectively. The suppressive effects in one month treatment gradually increased with dose-dependent manner, although suppressive effects were not high.

The results showed that galangin was effective in suppressing the frequencies of micronuclei induced by MNNG. Our study indicates that galangin is a potent anticlastogenic agent against MNNG.

Keywords □ galangin; anticlastogenic effect; micronucleus assay; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

Galangin, the flavone derivative having 3,5,7-trihydroxyl groups, is one of flavonoid compounds which distributed widely in the plant kingdom (Kuhnau, 1976). Galangin inhibited the clastogenicity of benzo(a)pyrene (B(a)P) in mouse bone-marrow micronucleus assay. Galangin also showed a stronger anticlastogenic effect than (−)-epicatechin, flavan-3-ol derivative, against ethyl methane sulfonate and 7,12-dimethyl benzo(a)anthracene as well as B(a)P (Heo *et al*, 1992). On the other hand, it has been reported that galangin had strong inhibition (97%) of the mutagenicity of 2-aminoanthracene on Salmonella typhimurium, TA 98 (Wall *et al*., 1988). It was suggested that galangin has a suppressive activity to the several types of genotoxic chemicals. These anticlastogenic effects of galangin were founded to attribute from the direct inhibition of metabolism of B(a)P and the inhibition of binding between DNA and B(a)P metabolites (Kim *et al*, 1991). Thus, galangin may potentially act as a chemopreventive agent for several classes of chemical carcinogens.

In the present paper, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was used as positive mutagen. Monofuctional alkylating agents such as MNNG are both mutagenic and carcinogenic in a variety of organism (Mandell and Greenberg, 1960; Schoenthal, 1966; Sugimura *et al*., 1970). Their occurence is widespread in the environment and human exposure from natural and pollutant source is universal. In our previous study, polyhydroxy flavonols including galangin were screened their anticlastogenic activity against MNNG-

* To whom correspondence should be addressed.

induced micronuclei using ICR mice. Galangin showed highest suppressive effect among polyhydroxy flavonols tested (Heo *et al.*, 1993). It might be very interesting to study more intensively the anticlastogenic activities of galangin toward MNNG-induced clastogenicity in order to develop galangin as a chemopreventive agent and as a model compound to evaluate the biological activities against direct acting genotoxic agents.

Therefore, we have investigated to elucidate the anticlastogenic effects of galangin against the induction of micronulei by MNNG in bone-marrow polychromatic erythrocytes of C57BL/6 inbred mice.

Materials and methods

Chemicals

Galangin (CAS No. 548-83-4) was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin, USA. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG, CAS NO. 70-25-7) was obtained from Fluka Chemical Company, Swizerland. Galangin was dissolved in corn oil. MNNG was dissolved in dimethyl sulfoxide (DMSO). In all experiments, the administration volume to mice was 0.1 m*l*/25 g orally or intraperitoneally.

Animals

Male C57BL/6 (15-20 g) 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± 14* and relative humidity of 55± 7% on the standard diet and water *ad libitum.*

Mouse Bone-marrow Micronucleus assay

Mice were treated intraperitoneally with different doses (0, 100, 150, and 200 mg/kg) for double administration with 24 hr interval to elucidate a dose-dependent effect of MNNG. In order to know an anticlastogenic activity against MNNG-induced micronuclei in bone-marrow cells of mice, galangin (0, 0.1, 1, and 10 mg/kg) was administered orally twice with 24 hr interval. MNNG was administered immediately and intraperitoneally after the dose of galangin. To know the effects of multiple pre-treatment, galangin was administered orally every day for one week or 5 days a week for one month. MNNG also was administered intraperitoneally and immediately after two last doses of galangin. Mice were treated with corn oil or DMSO as vehicle controls. The treated mice were sacrificed at 32 hr 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 statisical analysis, Student's t-test and Analysis of Variance test were used to compare the galangin-treated groups with the untreated group.

Results

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 hr interval. Mice were sacrificed at 32 hr after first dose of MNNG for micronucleus assay. As shown in Fig. 1, the frequencies of MNPCEs showed a dose-dependent increase in the double treatment. The highest frequency of MNPCEs in mouse bone-marrow cells showed in the double dose of 200 mg/kg body weight, but half number of mice treated died of high dose. Therefore, a double dose of 150 mg/kg was chosen to evaluate an anticlastogeicity of galangin.

In the subsequent experiment, galangin was orally administered at 0, 0.1, 1 or 10 mg/kg body weight with a concomitant injection of MNNG. Galangin decreased the frequency of induced MNPCEs at all doses tested, even at 0.1 mg/kg (Table I). However, the suppressive effect in the group of 10 mg/kg decreased slightly to compare with the group of 1mg/kg, although they were still lower than the group of MNNG alone. When galangin was administered at 10 mg/kg without MNNG as a negative control experiment, galangin did not any clastogenic activity.

When galangin was multiply administered at 1/7 or

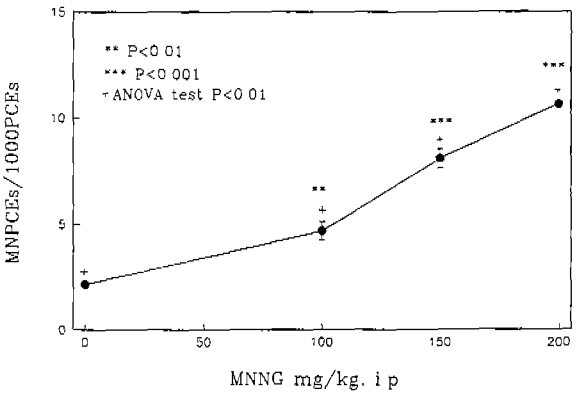

Fig. 1. Effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine concentration on MNPCEs in bone-marrow cells of C57BL/6 mice. Each point represents the mean± SE.

Table I. Suppression of MNNG-induced MNPCEs by simultaneous treatment of galangin in bone-marrow cells in C57BL/6 mice

MNNG[a] (mg/kg, i.p.)	Galangina (mg/kg, p.o.)	No. of mice	MNPCEs/1000 PCEs[b] mean± SE	PCE/NCE mean± SE	%[c] Suppression[c]
0	0	5	1.78± 0.37	1.14± 0.06	
0	10	5	0.79± 0.37	1.03± 0.07	
150	0	5	8.27± 0.38[d]	0.63± 0.07	
150	0.1	5	6.27± 0.86[d,*]	0.68± 0.09	24.2
150	1	5	2.94± 1.53[d,**]	0.50± 0.10	64.5
150	10	5	5.27± 1.55[d,**]	0.44± 0.03	36.3

[a]Galangin was administered orally twice with 24 h interval, and immediately MNNG was injected intaperitoneally after the dose of galangin.
[b]1000 PCEs were scored per animal.
[c]% Suppression was calculated by using the following formular.

$$\% \text{ Suppression} = 100 - \frac{\text{MNPCEs/1000PCEs in the presence of galangin}}{\text{MNPCEs/1000PCEs in the absence of galangin}} \times 100$$

[d]Significant dose-dependent decrease of MNPCEs ($P<0.01$; Analysis of Variance).
*$P<0.05$, **$P<0.01$; Significant induction of MNPCEs compared to the control group by Student's t-test.

Table II. Suppression of MNNG-induced MNPCEs by multiple pretreatment of galangin in bone-marrow cells in C57BL/6 mice

MNNG[a] (mg/kg,i.p.)	Galangin[a] (mg/kg, p.o.)	Period of treatment	MNPCEs/1000 PCEs[b] mean± SE	PCE/NCE mean± SE	%[c] Suppression[c]
150	0	–	8.27± 0.38	0.63± 0.07	
150	1/7	7 days	5.52± 0.97*	0.47± 0.04	23.5
150	1	7 days	7.15± 0.97	0.62± 0.04	13.5
0	1	7 days	1.74± 0.48	1.20± 0.09	
150	0.001	1 month	8.69± 1.53	0.57± 0.03	−5.1
150	0.01	1 month	8.22± 2.00	0.63± 0.11	5.5
150	0.1	1 month	6.66± 1.29*	0.43± 0.04	19.5

[a]Galangin was administered orally every day for 1 week or 5 days a week for 1 month. MNNG was injected intaperitoneally after two last dose of galangin.
[b]1000 PCEs were scored per animal. All data indicate mean SE of at least three mice per group.
[c]same as in Table I.
*$P<0.05$; Significant induction of MNPCEs compared to the control group by Student's t-test.

1 mg/kg for 7 days respectively, galangin showed higher suppressive effect in the group of 1/7 mg/kg than in the group of 1 mg/kg (Table II). As a control experiment, galangin (1 mg/kg) was administered for 7 consecutive days without MNNG injection to evaluate clastogenic activity of this compound. There was no clastogenic effect to compare with solvent control. In another experiment, galangin was administered at 0.001, 0.01 and 0.1 mg/kg for 1 month respectively. The suppressive effects in the group of 1 month treatment gradually increased with a dose-dependent manner. However, galangin showed a significant suppressive effect in the dose of 0.1 mg/kg only.

Discussion

It is well established that minor dietary constituents such as flavonoids could show protective effects against experimental carcinogenesis/mutagenesis (Huang, 1983; Nixon *et al.*, 1984; Francis *et al.*, 1989). The average daily intake of all flavonoids by human is estimated to be about 1 g per day (Herman, 1976). In our previous study, 14 flavonoids including galangin, showed an anticlastogenic activities against B(a)P-induced MNPCEs in mice. Among of them, galangin showed a potent anticlastogenic effect(Heo *et al*, 1992). These activity of galangin against B(a)P-induced MNPCEs in mice might be, partly at least, due to the direct inhibition of arylhydrocarbon hydroxylase and DNA-B(a)P metabolite adduct formation (Kim *et al.*, 1991).

In this study, our results indicate that the MNPCEs induced by MNNG in bone-marrow cells of C57BL/6 mice were also suppressed by simultaneous treatment or multiple pre-treatment of galangin. When galangin

was orally administered at 0, 0.1, 1.0, or 10.0 mg/kg twice with 24 hr interval, together with intraperitoneally administered MNNG, there were suppressive effects in the tested doses (Table I). The most marked suppressive effect was observed in the treatment group of 1.0 mg/kg (64.5%), not in the treatment group of 10.0 mg/kg(36.3%).

When galangin was multiply administered at 1/7 or 1 mg/kg for 7 days respectively, galangin showed higher suppressive effect in the treatment group of 1/7 mg/kg (23.5%) rather than in the treatment group of 1 mg/kg (13.5%)(Table II). Although suppression was observed with MNNG doses, the suppressive effect was more pronounced at low dose. It was similar to simultaneous treatment which showed higher suppressive effect in the treatment group of 1 mg/kg (64.5%) than in the treatment group of 10 mg/kg (36.3%). Based on these results, it is assumed that an anticlastogenicity of galangin depend on its administrative dose, and this could be due to the toxicity of galangin at cumulative dose.

In another experiment, galangin was administered at 0.001, 0.01 and 0.1 mg/kg for 1 month respectively. The suppressive effects in the multiple pre-treatment of 1 month gradually increased with dose-dependent manner (Table II). Since administrative doses of galangin tested were too low, galangin showed a significant suppressive effect in the dose of 0.1 mg/kg only.

In our recent study, polyhydroxy flavonols including galangin have been screening their anticlastogenic activity against MNNG-induced micronuclei using ICR mice. Most of flavonols tested were effective in suppressing the frequencies of micronuclei induced by MNNG (Heo *et al.*, 1993). We have not yet determined the mechanism which can explain the suppression of galangin on MNNG-induced MNPCEs. 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). Electrophilic 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 galangin may have a scavenging effect to react with alkonium ion or protect to bind between DNA and alkonium ions, although possibilities of other mechanisms such as enhancement of DNA-repair system could not be excluded.

Wall *et al.* (1988) reported an antimutagenic effect of galangin that examined in the salmonella typhimurim strain treated with 2-aminoanthracene, and 2-aminoanthracene in combination with galangin. We also show that galangin is a potent anticlastogen against MNNG in an *in vivo* mouse bone-marrow micronucleus assay. All of our results could suggest that galangin may be useful as a chemopreventive agent of MNNG. Therefore, galangin may have capable of protecting carcinogensis. And more intensive research is required to find out the action mechanisms of galangin against various genotoxic agents.

Acknowledgements

This research was supported by the grant from the New Drug Development Program, Ministry of Health and Social Affairs, Republic of Korea. and in part by grant 911-0406-012-2 from the Korea Science and Engeneering Foundation.

References

Francis, A. R., Shethy, T. K. and Bhattacharya, R. K. (1988). Modifying role of dietary factors on the mutagenicity of aflatoxin B_1: *In vitro* effect of plant flavonoids. *Mutation Res.* **222**, 393-401.

Gebhart, E. (1978). The anticlastogenic effect of various combinations of cysteamine, AET, HCT, and amino acids on chromosome damage by trenimon and bleomycin in human lymphocytes *in vitro*. *Hum. Genet.* **43**, 185-203.

Heo, M. Y., Yu, K. S., Kim, K. H., Kim, H. P. and Au, W. W. (1992) Anticlastogenic effects of flavonoids against mutagen induced micronucei in mice. *Mutation Res.* **284**, 243-249.

Heo, M. Y., Kwon, C. H., Sohn, D. H., Lee, S. J., Kim, S. W., Kim, J. H. and Au, W. W. (1993). Effects of flavonol derivatives on the micronuclei formation by *N*-methyl-*N'*-nitro-*N*-nitroguanidine and the enhancement of bleomycin-induced chromosome aberrations by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. *Arch. Pharm. Res.*, **16**, 196-204.

Herman, K. (1976). Flavonols and flavones in food plant: A review. *J. Food Toxicol.* **11**, 443-448.

Huang, M. T., Wood, A. W., Newmark, H. L., Sayer, J. M., Yagi, H., Jerina, D. M. and Conney, A. H. (1983). Inhibition of the mutagenicity of bay region diol epoxides of polycyclic aromatic hydrocarbons by plant flavonoids. *Carcinogenesis* **4**, 1631-1637.

Kim, H. K., Kim, K. H., Heo, M. Y. and Kim, H. P. (1991). Effect of antimutagenic flavonoid, galangin on benzo(a)pyrene metabolism in mice. *Korean Biochem. J.* **24**, 141-147.

Kuhnau, J. (1976). The flavonoids, a class of semi-essential food components, their role in human nutrition. *Wld. Rev. Nutr. Diet,* **24**, 117-191.

Loveless, A. (1969). Possible relevance of O^6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. *Nature* (London) **254**, 206-207.

Magee, P. N., Montesano, R. and Preussmann, R. (1976). *N*-nitroso compounds and related carcinogens. *Am. Chem.*

Soc. Monograph. Vol.173, American Chemical Soc., Washington, DC., pp. 491-625.

Mandell, J. D. and Greenberg, J. (1960). A new chemical mutagen for bactera, 1-methyl-3-nitro-1-nitrosoguanidine, *Biochem. Biophys. Res. Commum.* **3**, 575-577.

Nicoll, J. W., Swann, P. F. and Pegg, A. E. (1975). Effect of dimethyl nitrosamine on persistence of methylated guanines in rat liver and kidney DNA. *Nature* (London) **254**, 261-262.

Nixon, J. E., Hendricks, J. D., Pawlowski, N. E., Percera, C. B., Sinnhuber. R. O. and Bailey, G. S. (1984). Inhibition of aflatoxin B_1, *Carcinogenesis* **5**, 615-619.

Schmid, W. (1975). The micronucleus test. *Mutation Res.* **31**, 9-15.

Schoenthal, R. (1966). Carcinogenic activity of *N*-methyl-*N'*-nitro-*N*- nitroso guanidine. *Cancer Res.* **30**, 455-465.

Stubbe, J. and Kozarich, J. W. (1987). Mechanisms of bleomycin-induced DNA degradation. *Chem. Rev.* **87**, 1107-1136.

Sugimura, T., Fujimura, S. and Baba, T. (1970). Tumor production in the glandular stomach and alimentary tract of the rat by *N*-methyl-*N'*-nitro-*N*- nitrosoguanidine. *Cancer Res.* **30**, 455-465.

Wall, M. E., Wani, M. C., Manikumar, G., Arraham, P, Taylor, H., Hughes, T. J., Warner, J. and McGivney, R. (1988). Plant antimutagenic agents, Flavonoids. *J. Nat. Prod.* **51**, 1084-1091.