Antimutagenic Effect of Genistein

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Abstract

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A great variety of health benefits including the protection against breast and prostate cancers has been attributed to the soya consumption, because of the presence of soy beans isoflavones, genistein, and others. We investigated the antigenotoxic effect of genistein on the genotoxicity of three mutagens and carcinogens – aflatoxine B_1 (AFB_1), 2-amino-3-methylimidazo [4,5-f]quinoline (IQ), and *N*-nitroso-*N*-methylurea (MNU), using the Ames bacterial mutagenicity test and the micronucleus test. In the Ames test on *Salmonella typhimurium*, a significant antimutagenic effect was determined against the indirect mutagen AFB_1 in two strains, TA98 and TA100. However, the effect on the IQ indirect mutagenicity was more pronounced in the test with TA98 than with TA100. The mutagenicity of the direct mutagen MNU was suppressed by genistein only at its highest concentration used (300 μg/plate). The protective effect of genistein against all three mutagens was proved in the micronucleus test as the treatment of mice with the combinations of genistein and mutagens resulted in a significant reduction of the number of micronuclei in comparison with the number of micronuclei induced by the individual mutagens alone.

Keywords: chemoprevention; aflatoxin B_1; 2-amino-3-methylimidazo [4,5-f]quinoline; *N*-nitroso-*N*-methylurea; Ames test; micronucleus test

Genistein is a phytoestrogen belonging to the group of isoflavons with a wide variety of pharmacological effects. Genistein is synthetised in plants from flavanone naringenin. The major dietary source of genistein are soya products (Dixon & Ferreira 2002).

There exist numerous data showing the protective effects of soya products in animal models and in epidemiological studies (Rosenberg Zand *et al.* 2002). A cross-national study involving 50 countries identified soya products as foods with a protective effect against prostate cancer (Herbert *et al.* 1998). The low incidence of breast cancer in Asian women compared to women in western countries was attributed to a high consumption of soya products (Adlercreutz *et al.* 1991; Lee *et al.* 1991). This correlation is less evident among the second generations of Asian immigrants to USA adopting a western style diet (Ziegler *et al.* 1993). Urinary levels of soya derived isoflavones

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including genistein were lower in breast cancer patiens as compared with controls (Zheng *et al.* 1999). An association between high soya intake and lower incidence of endometrium cancer were also described (Goodman *et al*. 1997). Epidemiologic data are summarised in Park and Surh (2004). In a study of Lamartiniere *et al*. (2002), genistein was confirmed as an agent protecting animals against experimentally induced mammary and prostate cancers.

The antiestrogenic activity of genistein is the probable mechanism of its chemopreventive effect. Genistein shares the structural similarity with estrogen estradiol 17 β and the ability to be tied up with estrogen receptors. Thus, genistein exerts both estrogenic and antiestrogenic activities, the latter one by competing for estradiol receptors. The opposite effects are attributed to different responses provoked by different doses of genistein, which in lower concentrations exerts estrogen-like activity, in higher concentrations may act as an antiestrogen and an inhibitor of the tumour-promoting effects of estrogens (Sarkar & Li 2002, 2004).

Genistein has been shown to be an inhibitor of several intracellular enzymes such as tyrosin kinases, topoisomerase II, phosphatidylinositol kinases, ABC transporters, where genistein ligates their ATP-binding domain, thus involving cell signalling cascades and cell cycle progression. These effects on the molecular and cellular levels are summarised in Polkowski and Mazurek (2000); Sarkar and Li (2002, 2004); Park and Surh (2004). On the cellular level, it induces cellular differentiation, alters cell cycle progression, inhibits cell proliferation, and induces apoptosis. Genistein has an antiangiogenic effect, inhibits proteins involved in the multidrug resistance of cancer cells. Genistein exerts an antioxidant effect, protects cells against the reactive oxygen free radicals, and inhibits osteoclastic function.

There also exist information on genotoxicity of genistein detected by studies *in vivo* and *in vitro* using higher concentrations (for review see Stopper *et al.* 2005).

Messina and Loprinzi (2001), while reviewing the literary data, came to uncertainity that the comsumption of soya may affect the risk of breast cancer or the survival of breast cancer patiens. Because of this conflicting results, genistein is under intensive study.

In our work, we present the effect of genistein on the mutagenicity of two indirect mutagens, i.e. aflatoxin B_1 (AFB_1) and 2-amino-3-methylimidazo [4,5-f]quinoline (IQ), and on the mutagenicity of the direct mutagen *N*-nitroso-*N*-methylurea (MNU), using the Ames test *in vitro,* and the micronucleus test *in vivo*.

MATERIAL AND METHODS

The Ames test

For the evaluation of the antimutagenic effect of genistein *in vitro*, the Ames test was performed using *Salmonella typhimurium* TA98 and TA100 strains (Ames 1971; Ames *et al.* 1975; Maron & Ames 1983; Černá *et al*. 1989).

The mutagenic substances were used at the following concentrations: AFB_1 at the concentrations of 10 μg, 1 μg, and 0.1 μg per plate in both strains, TA98 and TA100; IQ at the concentrations of 0.1 μg, 0.01 μg, and 0.001 μg per plate in the strain TA98, and at the concentrations of 10 μg, 1 μg, and 0.1 μg in the strain TA100; MNU at the concentrations of 1000 μg, 100 μg, and 10 μg in the strain TA100 only. These MNU concentrations had no effect upon the strain TA98. Each concentration of the individual mutagens was combined with four different concentrations of genistein (300 μg, 30 μg, 3 μg, and 0.3 μg per plate). All chemicals were diluted in DMSO. For the metabolic activation the S9 liver homogenate fraction from laboratory rats treated with a mixture of polychlorinated biphenyls Delor was used (Maron & Ames 1983). All combinations of the mutagens and antimutagen were tested in two separate experiments, with three plates in each experiment.

The percentage of the inhibition of mutagenicity was calculated using the following formula:

[(No. of revertants of mutagen – No. of revertants of mixture of mutagen and genistein)/No. of revertants of mutagen] × 100

The micronucleus test

The experiments *in vivo* (bone marrow micronucleus test) were carried out on ten-week-old male Balb C mice, weighing 22–26 g. The animals were housed under standard conditions in groups of 10 mice for the treatment.

The following concentrations of mutagens were used for *in vivo* tests: AFB_1 5 mg/kg of body weight (b.w.), IQ 20 mg/kg b.w., MNU 50 mg/kg b.w.

Genistein was administered at the dose of 20 mg per kg b.w. to mice by gavage for three consecutive days. Carcinogens were administered at one dose on the third day. All the substances (diluted in DMSO) were administered in the volumes of 100 µl/10 g b.w. The control mice were orally treated with 7% solution of DMSO.

The mouse bone marrow micronucleus test was carried out according to Schmid (1975). A total of 1000 polychromatophilic erythrocytes were scored per animal by the same observer for evaluating the frequencies of micronucleated polychromatophilic erythrocytes. Each experiment was run three times.

The statistical significance of the differences between two means defined for the respective mutagen and its mixture with genistein was tested by the Student's *t*-test.

RESULTS

The results of the Ames test (Tables 1–3) are expressed as a number of revertants, and also as the percentage of inhibition of the mutagen activity of the sample consisting of a mixture of the respective mutagen and genistein in comparison with the mutagenicity of the individual mutagen, according to the formula presented in Methods.

The number of revertants induced by genistein did not differ from the control values. Two highest concentrations of genistein (300 and 30 µg per plate) revealed a significant dose – dependent antimutagenic effect upon all concentrations of AFB_1 in the TA98 and TA100 strains. The lower concentration of genistein, 3 µg per plate, was significantly antimutagenic only in combination with 1 and 0.1 µg of AFB_1 per plate in both strains (Table 1).

Table 1. Effect of genistein on mutagenicity of AFB_1 – Ames test

AFB_1 + genistein dose (µg/plate)	*S. typhimurium* TA98 + S9			*S. typhimurium* TA100 + S9		
	No of revertants	± SD	% of inhibition	No of revertants	± SD	% of inhibition
10 + 0	807	113		989	50	
10 + 0.3	784	104	–3	937	50	–5
10 + 3	733	118	–9	916	52	–7
10 + 30	519**	51	–36	815**	71	–18
10 + 300	182**	16	–77	310**	98	–69
1 + 0	254	45		567	36	
1 + 0.3	231	28	–9	534	27	–6
1 + 3	185*	19	–27	498*	32	–12
1 + 30	124**	14	–51	322**	34	–43
1 + 300	47**	7	–81	118**	21	–79
0.1 + 0	79	10		212	14	
0.1 + 0.3	81	15	+3	186	31	–12
0.1 + 3	57**	2	–28	161**	11	–24
0.1 + 30	45**	4	–43	129**	12	–39
0.1 + 300	29**	3	–63	103**	10	–51
Control – DMSO	25	3		95	8	
0 + 0.3	24	5		103	6	
0 + 3	23	3		101	6	
0 + 30	24	3		106	12	
0 + 300	27	4		94	11	

SD – standard deviation; *$P \leq 0.05$; **$P \leq 0.01$

Table 2. Effect of genistein on mutagenicity of IQ – Ames test

IQ + genistein dose (μg/plate)	*S. typhimurium* TA98 + S9			IQ + genistein dose (μg/plate)	*S. typhimurium* TA100 + S9		
	No. of revertants	± SD	% of inhibition		No. of revertants	± SD	% of inhibition
0.1 + 0	1113	98		10 + 0	1142	206	
0.1 + 0.3	1022	87	–8	10 + 0.3	1125	350	–1
0.1 + 3	1019	102	–8	10 + 3	964	215	–16
0.1 + 30	953*	27	–14	10 + 30	964	280	–16
0.1 + 300	491**	66	–56	10 + 300	549*	354	–52
0.01 + 0	387	72		1 + 0	380	128	
0.01 + 0.3	370	77	–4	1 + 0.3	370	85	–3
0.01 + 3	338	65	–13	1 + 3	349	113	–8
0.01 + 30	249**	43	–36	1 + 30	345	122	–9
0.01 + 300	117**	15	–70	1 + 300	171*	62	–55
0.001 + 0	114	23		0.1 + 0	155	42	
0.001 + 0.3	96	21	–16	0.1 + 0.3	147	39	–5
0.001 + 3	71**	15	–38	0.1 + 3	157	44	+1
0.001 + 30	63**	11	–45	0.1 + 30	131	29	–15
0.001 + 300	34**	5	–70	0.1 + 300	106	32	–32
Control – DMSO	25	4		Control – DMSO	106	32	
0 + 0.3	27	5		0 + 0.3	113	16	
0 + 3	27	4		0 + 3	108	29	
0 + 30	24	4		0 + 30	114	30	
0 + 300	26	4		0 + 300	106	35	

SD – standard deviation; *$P \leq 0.05$; ** $P \leq 0.01$

The effect of genistein on IQ mutagenicity was more pronounced in the tests with TA98 strain than in those with TA100 strain. Two highest genistein concentrations were antimutagenic in combinations with all doses of IQ in TA98, while the concentration of 3 μg per plate only in combination with the lowest IQ dose (0.001 μg per plate). The effect was dose dependent. However, in the strain TA100 only the highest concentration (300 μg per plate) significantly suppressed the mutagenicity of 10 and 1 μg of IQ per plate (Table 2).

The mutagenicity of the direct mutagen MNU was suppressed by 300 μg of genistein per plate in combinations with 100 and 10 μg of MNU per plate in the strain TA100. Other concentrations of genistein were without the antimutagenic effect (Table 3).

In the micronucleus tests all three mutagens revealed significant mutagenic activities. The number of micronuclei in animals influenced by genistein alone did not differ from that of the control group. Oral administration of the combination of genistein (at a dose of 20 mg/kg b.w.) and aflatoxin B_1 revealed a lower number of micronuclei in polychromatophilic erythrocytes in a statistically significant degree in comparison with laboratory mice treated with AFB_1 alone.

Similarly, a significant suppression of IQ mutagenicity was reached with the combination of genistein and the IQ mutagen.

The treatment of mice with the combination of genistein and MNU resulted in a significant reduction of the number of micronuclei in comparison with the number of micronuclei induced

Table 3. Effect of genistein on mutagenicity of MNU – Ames test

MNU + genistein dose (µg/plate)	*S. typhimurium* TA100		
	No. of revertants	± SD	% of inhibition
1000 + 0	1726	240	
1000 + 0.3	1775	115	+3
1000 + 3	1746	139	+1
1000 + 30	1699	136	–2
1000 + 300	1350	420	–22
100 + 0	1811	122	
100 + 0.3	1866	153	+3
100 + 3	1883	157	+4
100 + 30	1758	229	–3
100 + 300	1334**	138	–26
10 + 0	1050	262	
10 + 0.3	1011	290	–4
10 + 3	1021	302	–3
10 + 30	932	244	–11
10 + 300	598*	336	–43
Control – DMSO	110	8	
0 + 0.3	107	15	
0 + 3	117	14	
0 + 30	108	7	
0 + 300	98	11	

SD – standard deviation; *$P \leq 0.05$; **$P \leq 0.01$

by MNU alone. The results of the micronucleus test are presented in Table 4.

Table 4. Efect of genistein on mutagenicity of AFB_1, IQ and MNU – micronucleus test

Substance studied	Number of micronuclei	± SD
Control 7% DMSO	2.0**	0.82
Genistein 20 mg/kg	2.6**	0.49
AFB1 5 mg/kg	8.2*	2.5
Genistein + AFB1 20 mg/kg + 5 mg/kg	4.0**	1.6
IQ 20 mg/kg	11.0*	2.45
Genistein + IQ 20 mg/kg + 20 mg/kg	3.6**	1.62
MNU 50 mg/kg	21.8*	5.2
Genistein + MNU 20 mg/kg + 50 mg/kg	9.2**	2.9

*significantly higher number of micronuclei as against the negative control (DMSO)
**significantly lower number of micronuclei as against mutagen alone
SD – standard deviation

DISCUSSION

It is well known that damage to the genome or aberrant DNA methylation, resulting in aberrant gene expression (suppression of tumour suppressor genes and inappropriate expression of oncogenes), is fundamental to tumorigenesis. The variability in cancer expression is due to the differences in the amount of DNA damage and the capacity to repair DNA damage, both being influenced by the genetic predisposition (gene polymorphism) and the dietary factors. Metabolism including the detoxification of genotoxic chemicals is influenced by the dietary factors, and the dietary intervention offers us a good oportunity for the cancer prevention.

Many protective compounds were discovered in plants; among them, genistein has been extensively investigated for its chemopreventive ability, especially against tumours of breast and prostate. Its effect involves antioxidant properties, modulation of key enzymes and inhibitors of the cell cycle (Choi *et al.* 1998a, b), and induction of apoptosis in transformed cells (Kumi-Kiaka *et al*. 2000; Sarkar & Li 2004).

On the other hand, there are also studies on genotoxicity of this compound. In experimental animals, Dingley *et al.* (2003) detected an increase of PhIP (2-amino-1-methyl-6 phenyl-imidazo[4,5-b] pyridine) adducts but not IQ adducts after genistein treatment. In the study by Tsutsui *et al*. (2003) genistein was shown to induce chromosomal aberrations, aneuploidies, DNA adducts, and transformation of Syrian hamster embryonal cells.

Misra *et al.* (2002) described a significant increase in the frequency of micronucleated erythrocytes, but this effect was small and not dose related; genistein had no effect on the incidence of tumours developed in p53 knockout mice. Misra did not prove the genotoxic effect in the Ames test without metabolic activation; after metabolic

activation, only a small increase in the number of revertants was detected in TA100 strain of *Salmonella typhimurium*, however, not in other tester strains.

We did not detect any genotoxic effect of genistein either in the Ames test or in the micronucleus test, because the number of revertants or the number of micronuclei did not differ significantly from the controls. We proved a clear dose-dependent antimutagenic effect of genistein upon the indirect mutagenicity of AFB_1 and IQ in the Ames test. The effect upon AFB_1 mutagenicity was similar in TA98 and TA100 strains. The effect upon IQ mutagenicity was stronger in TA98 strain than in TA100 strain. The direct mutagenicity of MNU was suppressed only by the highest concentration of genistein in TA100 strain. Clear antimutagenic effects of genistein upon the mutagenicity of all three mutagens were also proved in the micronucleus test.

Similar results were achieved by Weisburger *et al*. (1998) in the Ames test, a dose-related inhibition of the mutagenicity of heterocyclic amine PhIP by genistein was detected. Miyazava *et al*. (1999) proved antimutagenic activity of genistein against Trp-P-1 (3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole) and furylfuramide in the Ames and umu tests.

Antigenotoxic effect of genistein and also its opposite effect are discussed in excellent reviews by Polkowski and Mazurek (2000), Sarkar and Li (2002) and Park and Surh (2004), altogether with the explanation of its effects at molecular and cellular levels.

It is supposed that genotoxicity of genistein can be caused by the inhibition of DNA topoisomerase II resulting in stabilisation of DNA double strand breaks at topoisomerase II-DNA binding sites (Boos & Stopper 2000; Snyder & Gillies 2002; Stopper *et al*. 2005).

It is evident that the understanding of the effects of genistein and other phytoestrogens is far from being clear. Their bipolar response – protective in lower doses and possible genotoxicity of higher doses, especially in *in vivo* experiments, needs additional studies in this field. It is also obvious that the concentrations necessary for the genotoxic effect in *in vitro* studies and in experimental studies *in viv*o can hardly be reached by our usual diet. But we must be carefull with the use of phytoestrogen concentrates in menopause. Phytoestrogens are often presented as non-hormonal. This may be dangerous for the women under the risk of re-occurrence of estrogen dependent cancers. In addition, the review of 105 clinical studies has not brought any clear proof that phytoestrogens lower the risk of breast cancer and cardiovascular diseases, but it seems to be evident that they decrease the risk of osteoporosis (Cornwell *et al*. 2004).

Since phytoestrogen genistein has a lower afinity to the estrogen receptors than the physiological ligand estradiol, it may act as an enhancer of the cell proliferation in the absence of hormone, but may be anti-estrogenic in the presence of estradiol and reduce estradiol-mediated cell proliferation. Interactions of different compounds with genotoxic and antigenotoxic effects may change the final effect of the individual compounds. Additionally, other environmental or life style factors may be related to the risk of cancers (Bouker & Hilakivi-Clarke 2000).

The reviewed data indicate that the intake of concentrated phytoestrogens as supplements should not be advised to menopausal women while diet containing high amounts of plant substances is important for the health maintenance (Stopper *et al*. 2005).

Further studies are needed on the effects of genistein and other phytoestrogens, especially studies on the combined effects of different plant substances, because of the possible interactions of genotoxic and antigenotoxic compounds. In a diet, this interactions may result in potentiation, as well as in antagonistic effects.

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