Antimutagenic Effect of Ellagic Acid and its Effect on the Immune Response in Mice

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Abstract

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Using the Ames bacterial mutagenicity test and an *in vivo* micronucleus test, we investigated the antigenotoxic effect of ellagic acid on the genotoxicity of three mutagens: amino-methylimidazo-quinoline (IQ), aflatoxin B_1 (AFB_1), and *N*-nitroso-*N*-methylurea (MNU). Ellagic acid is a naturally occurring phenolic compound which is found in a variety of soft fruits and vegetables. The effect of this compound on the immunosuppressive activity of mutagens was followed *in vivo* by the chemiluminescence test. In the Ames assay, ellagic acid at concentrations of 300 and 30 µg/plate demonstrably inhibits the mutagenic activity of two indirect mutagens: IQ and AFB_1. The concentration of 300 µg/plate had the strongest effect on mutagenicity of all concentrations of IQ in strain TA98 of *Salmonella typhimurium*, whereas in strain TA100 concentration of 30 µg per dish of ellagic acid was more effective than 300 µg per plate. Also in combination with different concentrations of AFB_1, ellagic acid proved to be a strong antimutagen. In this case the lower of the two effective concentrations – 30 µg/plate – had a much greater antimutagenic effect on both strains tested than 300 µg/plate. In combination with the direct mutagen MNU, ellagic acid did not show any marked antimutagenic effect at most of the concentrations tested in strain TA100. Only the highest concentrations of ellagic acid reduced the mutagenic effect of MNU weakly and only in combination with two lower concentrations of MNU. In the micronucleus test, three-day oral application of ellagic acid prior to the applicaton of AFB_1, IQ, or MNU, respectively, markedly reduced the numbers of micronuclei induced by these three mutagens in polychromatophilic erythrocytes of mice. Chemiluminescence test with mouse granulocytes proved that ellagic acid not only prevents the inhibitory effects of mutagens on free oxygen radicals and hydrogen peroxide production, but that this production is stimulated by ellagic acid in combination with mutagens even to a greater extent than by ellagic acid alone. From these results we can deduce that ellagic acid repairs strong immunosuppressive effects of all mutagens applied.

Keywords: ellagic acid; amino-methylimidazo-quinoline; aflatoxin B_1; *N*-nitroso-*N*-methylurea; antimutagenic activity; chemiluminescence

Tumors in man are largely the result of the action of environmental factors (SURH 1999). These environmental factors include chemical carcinogens and radiation originating almost exclusively from human activities. Besides carcinogenic substances anthropogenic in character, a group of natural carcinogens also exists. Among those are substances produced by microscopic fungi – moulds, as secondary metabolites called mycotoxins.

Aflatoxin B_1 (AFB_1) is one of the most thoroughly studied and a well known mycotoxin with carcinogenic activity. In the present study, it is used as a reference mutagen which effects mutagenic activity in all prokaryotic and eukaryotic testing systems (NIX *et al*. 1981; REDDY & SHARMA 1989; BÁRTA *et al.* 1990, 1997; MCQUEEN & WAY 1991; ŠMERÁK *et al*. 2001 and others) as well as immunosuppression (MICHAEL *et al*. 1973; CORRIER 1991).

Apart from substances contaminating foodstuffs, some substances may be formed through harsh processing procedures. From the point of view of mutagenicity, carcinogenicity or immunosuppressive activity, pyrolysates of

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amino acids (heterocyclic amines) of the category of amino-methylimidazo-quinoline occurring in human foodstuffs as a result of inappropriate heat processing of meat (LOPRIENO *et al*. 1991; ADAMSON *et al*. 1996) are considered to be the most serious. 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) in the isolated form has been used as another reference mutagen. The third reference mutagen in the present study is *N*-nitroso-*N*-methylurea (MNU) which is an important carcinogenic N-nitroso compound; in contrast to IQ and AFB_1 it is a directly acting carcinogen requiring no metabolic activation. This compound is all the more hazardous as it can be formed endogenously as demonstrated by SANDER (1971) and by MIRVISH *et al*. (1973). MNU participates in the induction of brain tumors and other tumors of nerve tissue, kidney, the urinary bladder, lungs, and stomach. It also plays a role in the mammary tumor formation as demonstrated in mice, as well as in the occurrence of thyroid gland tumors (IARC 1978).

On the other hand, certain bioactive components of plant foodstuffs can inhibit, retard, or even reverse individual steps of the process of carcinogenesis. The majority of such substances have in addition anti-oxidative and anti-inflammatory effects and can be applied, consequently; as effective chemopreventive or chemoprotective agents. While mutagenic and co-mutagenic substances may occur in foodstuffs as well as in the ambient air, antimutagens can act only through their intake in foodstuffs (HAYATSU *et al*. 1988).

Ellagic acid (EA), a dimer of gallic acid, belongs to the polyphenols. It is found mainly in fruit (strawberries, raspberries, grapes, black currant) as well as in various species of nuts. It has antimutagenic and anti-carcinogenic properties (VAN LIESHOUT *et al*. 1998; LOARCA-PINA *et al*. 1996, 1998; SURH 1999). Through its anti-oxidative effect (COZZI *et al*. 1995) and decreasing of cytochrome P450 activity (AHN *et al*. 1996), it reduces the metabolic activation of carcinogenic substances. It also increases the activity of glutathione-S-transferase and inhibits damage to DNA (BARCH *et al*. 1995). It markedly reduces the mutagenic activity of aflatoxin B_1 (LOARCA-PINA *et al*. 1996, 1998).

In the present paper, we studied the effect of ellagic acid on the mutagenicity of AFB_1, IQ, and MNU followed by the Ames test as well as by the micronucleus test, and its effect on natural immunity in mice followed by the chemiluminescence test.

MATERIAL AND METHODS

The Ames test. We evaluated the antimutagenic effect of ellagic acid on mutagens *in vitro* using the Ames test (AMES 1971; AMES *et al*. 1975; MARON & AMES 1983; ČERNÁ *et al*. 1989). The test was performed according to the standard methodology (ČERNÁ *et al*. 1989). The testing strains used in the present study were auxotrophic his bacterial strains of *Salmonella typhimurium* TA98 and TA100. S9 fraction of liver homogenate from laboratory rats induced by a mixture of polychlorinated biphenyls Delor was used for metabolic activation of indirect mutagens (IQ and AFB_1).

Mutagenic substances were applied at the following concentrations: IQ at concentrations of 0.1 μg, 0.01 μg and 0.001 μg per plate for strain TA98; at concentrations of 10 μg, 1 μg and 0.1 μg per plate for strain TA100; AFB_1 at concentrations of 10 μg, 1 μg and 0.1 μg per plate for both strains, TA98 and TA100; MNU at concentrations of 1000 μg, 100 μg and 10 μg per plate for strain TA100 only as these concentrations had no effect on strain TA98. Each concentration of the individual mutagens was combined with four different concentrations of ellagic acid (300, 30, 3, and 0.3 μg per plate). Each combination of a mutagen and ellagic acid was tested in two separate experiments with three plates in each experiment.

Effect of ellagic acid was expressed as percentage of inhibition of mutagenic activity following the formula:

$$\frac{\text{(number of revertants of mutagen – number of revertants of mutagen with antimutagen)}}{\text{number of revertants of mutagen}} \times 100$$

Experimental animals. All *in vivo* experiments (bone marrow micronucleus test and chemiluminescence test) were carried out on ten-week-old male Balb C mice, each weighing 22–26 g, purchased from BIOTEST (Konárovice, Czech Republic). These animals were divided into groups containing 7–10 mice. Both experiments were performed on the same animals.

Application of substances tested. Ellagic acid was applied to mice 3 times in one-day intervals at doses of 4 g/kg for the combination with AFB_1 or IQ, and at doses of 2 g/kg for the combination with MNU. The mutagens were applied in a single dose on the third day, 1–1.5 h after the application of ellagic acid, AFB_1 at the the dose of 1 mg/kg, IQ at the dose of 20 mg/kg, and MNU at the dose of 20 mg/kg. The substances tested were applied to mice *per os* by gavage. In all the experiments, each respective substance was dissolved in 7% DMSO and applied in volumes of 0.1 ml/10 g of murine body weight. Equal amounts of solvent (7% DMSO) were applied to the control group. All chemicals were purchased from Sigma.

The mutagenic effects of the substances and their mixtures were evaluated *in vivo* using of the micronucleus test (SCHMID 1975).

For the follow up of the effect of ellagic acid on the immune reaction of mutagen-treated mice, the chemiluminescence test (DE CHATELET *et al*. 1982; THOMAS *et al*. 1988; ŠESTÁKOVÁ *et al*. 1997) was used.

The micronucleus test. The micronucleus test is a method used for the detection of substances with a clastogenic effect, i.e. the capacity to induce the breakage of

chromosomes. An increased frequency of micronuclei in polychromatophilic erythrocytes in comparison with control groups indicates that the substance tested induces chromosomal damage in nucleated erythrocytes in the bone marrow. A total of 1000 polychromatophilic erythrocytes were scored per animal by the same observer for the evaluation of the frequencies of micronucleated polychromatophilic erythrocytes. Each experiment was run three times. For the statistical analysis, Student's *t*-test was used.

The chemiluminescence test. The importance of chemiluminescence tests lies in the possibility of following the influence of individual substances on the production of free radicals of oxygen in polymorphonuclear leucocytes. This capability influences the potential of an increased effect of inflammatory reactions and phagocytosis in relation to bacterial cells but it simultaneously increases the risk of the action of free radicals under an insufficient anti-oxidative protection. The induction of oxygen radicals is important in relation to the atherogenic as well as to the oncogenic processes.

The chemiluminescence test detects the production of free oxygen radicals, namely of hydrogen peroxide in the complex hydrogen peroxide – myeloperoxidase – halide cofactor. The chemiluminescence value reflects the degree of phagocytosis of zymosan particles by murine granulocytes. Four groups of mice were examined: a group treated with mutagen, a group treated with ellagic acid, a group treated with mutagen and ellagic acid, and an untreated control group. In all the groups of experiments performed, the reactions of granulocytic pooled cultures from 10 or at least 7 animals, were mutually compared. The time dependence of chemiluminescence values in each group of mice was followed and the maximum values of curves obtained are presented in figures.

The chemiluminescence test was performed according to the modification of ŠESTAKOVÁ *et al.* (1997). Polymorphonuclear leucocytes were obtained from the peritoneum of ten mice (per one test) four hours after the application of 5 ml glycogen. After the washing procedure, the final concentration of cells was adjusted to

Table 1. Effect of ellagic acid on mutagenicity of IQ – combination of EA + IQ with metabolic activation by S9 fraction of liver homogenate

S. typhimurium strain TA98 + S9				*S. typhimurium* strain TA100 + S9			
Dose (μg/plate) IQ + EA	number of revertants	SD	% inhibition	dose (μg/plate) IQ + EA	number of revertants	SD	% inhibition
0.1 + 0	1222	183		10 + 0	1076	189	
0.1 + 0.3	1360	98	–	10 + 0.3	1082	231	1
0.1 + 3	1223	98	–	10 + 3	866	295	20
0.1 + 30	538	209	56	10 + 30	108	17	90
0.1 + 300	343	82	72	10 + 300	155	38	86
0.01 + 0	583	136		1 + 0	578	207	
0.01 + 0.3	578	151	1	1 + 0.3	603	276	–
0.01 + 3	562	210	4	1 + 3	560	299	3
0.01 + 30	144	43	75	1 + 30	97	10	81
0.01 + 300	82	20	86	1 + 300	142	35	73
0.001 + 0	126	37		0.1 + 0	169	43	
0.001 + 0.3	143	55	–	0.1 + 0.3	180	58	–
0.001 + 3	131	26	–	0.1 + 3	152	60	10
0.001 + 30	54	10	57	0.1 + 30	100	10	41
0.001 + 300	48	7	62	0.1 + 300	120	26	29
Control (DMSO)	41	5		Control (DMSO)	95	5	
0 + 0.3	40	7		0 + 0.3	120	20	
0 + 3	37	5		0 + 3	106	7	
0 + 30	33	9		0 + 30	102	18	
0 + 300	43	7		0 + 300	135	23	

SD = standard deviation

5.75×10^6/ml in Hanks solution at pH 7.3. In each polystyrene tube were blended 0.4 ml Hanks solution, 0.1 ml dilute luminol (3.5 mg luminol in 2 ml DMSO is diluted 1:10 in redistilled water), 0.4 ml cell suspension, and 0.1 ml 1% zymosan as stimulant (0.1 ml Hanks solution in controls). Chemiluminescence activity was measured at room temperature at 5-min intervals over a period of 90 min in an analytical luminometer. Results are presented in maximum values (mV) of the chemiluminescence response of polymorphonuclear leucocytes in the dependence on time.

RESULTS

The Ames test. All the mutagens revealed a dose dependent mutagenic activity in the Ames test (Tables 1–3). The two highest doses of ellagic acid (EA), i.e. 30 and 300 µg/plate, showed an obvious antimutagenic activity in this test. In strain TA98, these two concentrations reduced the mutagenic activity of IQ (0.1 µg/plate) by 56 and 72%, respectively; the mutagenicity of 0.01 µg IQ per plate was decreased by 75 and 86%, respectively, the effect of the smallest dose of IQ (0.001 µg/plate) was inhibited by 57 and 62%, respectively. In strain TA100, the two highest concentrations of ellagic acid reduced the mutagenic activity of the individual doses of IQ as follows: the effect of 10 µg/ plate by 90 and 86%, 1 µg/ plate by 81 and 73%, and that of 0.1 µg/plate by 41 and 29%. In strain TA100, the concentration of 30 µg/plate had a greater effect on mutagenity of all concentrations of IQ than that of 300 µg/plate; in strain TA98, the most effective concentration was 300 µg/plate (Table 1).

A similar effect – a higher efficiency of 30 µg of ellagic acid than that of 300 µg/plate, was observed with all the tested concentrations of AFB_1 in both strains (Table 2). The mutagenicity of 10 µg AFB_1 was reduced, respectively, by 30 and 300 µg/plate of ellagic acid by 56 and 36% in TA98, by 57 and 34% in TA100; the activity of 1 µg AFB_1 by 63 and 54% in TA98, and by 73 and 57% in TA100. Mutagenicity of the lowest concentration of AFB_1, i.e. 0.1 µg/plate, was inhibited by 51 and 41% (TA98), and by 47 and 23% in TA100 in comparison with the activities of the respective doses of the mutagen alone. The antimu-

Table 2. Effect of ellagic acid on mutagenicity of AFB_1 – combination of EA + AFB_1 with metabolic activation by S9 fraction of liver homogenate

Dose (µg/plate) IQ + EA	*S. typhimurium* strain TA98 + S9			*S. typhimurium* strain TA100 + S9		
	number of revertants	SD	% inhibition	number of revertants	SD	% inhibition
10 + 0	1284	77		1573	74	
10 + 0.3	1214	83	6	1456	154	7
10 + 3	1060	122	17	1343	207	15
10 + 30	568	151	56	681	59	57
10 + 300	824	147	36	1033	213	34
1 + 0	825	179		931	191	
1 + 0.3	732	145	11	613	148	34
1 + 3	644	89	22	522	125	44
1 + 30	305	107	63	255	73	73
1 + 300	376	102	54	404	90	57
0.1 + 0	217	22		203	32	
0.1 + 0.3	222	26	–	197	27	3
0.1 + 3	239	11	–	165	21	19
0.1 + 30	106	16	51	107	11	47
0.1 + 300	129	15	41	157	9	23
Control (DMSO)	29	4		103	6	
0 + 0.3	31	3		105	11	
0 + 3	33	6		101	15	
0 + 30	31	3		88	4	
0 + 300	26	5		99	8	

SD = standard deviation

Table 3. Effect of ellagic acid on mutagenicity of MNU – *S. typhimurium* strain TA100

Dose (μg/plate) MNU + EA	Number of revertants	SD	% inhibition
1000 + 0	1414	51	
1000 + 0.3	1425	105	–
1000 + 3	1406	96	1
1000 + 30	1375	117	3
1000 + 300	1539	139	–
100 + 0	889	223	
100 + 0.3	903	189	–
100 + 3	989	189	–
100 + 30	789	195	11
100 + 300	672	185	24
10 + 0	261	41	
10 + 0.3	263	36	–
10 + 3	202	22	23
10 + 30	174	17	33
10 + 300	201	50	23
Control (DMSO)	80	6	
0 + 0.3	91	5	
0 + 3	92	9	
0 + 30	92	20	
0 + 300	94	12	

tagenic effect of the two lowest concentrations of ellagic acid (0.3 and 3 μg/plate) was not significant except for certain concentrations of AFB_1 (1 μg/plate) in TA98 and TA100.

The mutagenicity of the direct mutagen MNU at its highest concentration of 1000 μg/plate was not influenced by ellagic acid; only at lower concentrations (100 and 10 μg per plate) it was reduced in the range of 11–33%. The antimutagenic activity was generally lower than that against indirect mutagens and was the most effective at the lowest concentration of MNU, i.e. 10 μg/plate (Table 3).

The micronucleus test. No significant increase in the frequency of micronuclei in the control mice treated by DMSO was observed in comparison with intact animals. The number of micronuclei in animals influenced by ellagic acid alone did not differ from that of the control group. In the groups of animals treated with individual mutagens, a statistically significant higher number of micronuclei in polychromatophilic erythrocytes of the bone marrow was found in comparison with the control group.

On oral application of the combination of three doses of ellagic acid (4 g/kg) and a single dose of aflatoxin B_1 (1 mg/kg) the number of micronuclei in polychromatophilic erythrocytes was lower to a statistically significant degree in comparison with the laboratory mice treated with AFB_1 alone.

A similar effect was observed on application of the IQ mutagen: ellagic acid in combination with the IQ mutagen (3 × 4 g/kg + 20 mg/kg) reduced its mutagenic effect to a statistically significant degree. Similarly the treatment of mice with a combination of ellagic acid and MNU (3 × 2 g/kg + 20 mg/kg) led to a significant reduction of the number of micronuclei in comparison with the number of micronuclei induced by MNU alone. The results are presented in Table 4.

The chemiluminescence test. In all experiments, four groups of animals were examined: groups of animals treated with the mutagens, with ellagic acid alone, with their combinations, and the control group.

As to the effect of the combination of ellagic acid (4 g/kg) and AFB_1, in the group of mice treated with AFB_1, reduced chemiluminescence values (except for the first examination) were observed in comparison with the control group and the two other groups of mice ($P < 0.01$). Markedly elevated chemiluminescence values were found in the group of mice treated with a combination of AFB_1 and ellagic acid as compared to the control group ($P < 0.01$; 29th day, $P < 0.05$). In the group of mice treated with ellagic acid alone, a less marked increase in chemiluminescence was observed in comparison with the control group (3rd and 8th days, $P < 0.01$; 1st and 11th days, $P < 0.05$) (Fig. 1 and Table 5).

Table 4. Numbers of micronuclei in polychromatophilic erythrocytes in the bone marrow of mice treated with a combination of ellagic acid and the mutagens

Substance studied	Number of micronuclei	SD
Control (7% DMSO)	2.8	1.6
Ellagic acid (3 × 4 g/kg)	2.7	1.2
AFB_1 (1 mg/kg)	7.7*	2.2
AFB_1 + ellagic acid (1 mg/kg + 3 × 4 g/kg)	3.8**	2.4
IQ (20 mg/kg)	8*	2
IQ + ellagic acid (20 mg/kg + 3 × 4 g/kg)	4.9**	2
MNU (20 mg/kg)	20.6*	5.7
MNU + ellagic acid (20 mg/kg + 3 × 2 g/kg)	13.5**	2.7

* significantly higher number of micronuclei as compared with the negative control (DMSO)

** significantly lower number of micronuclei as compared with mutagen alone

SD = standard deviation

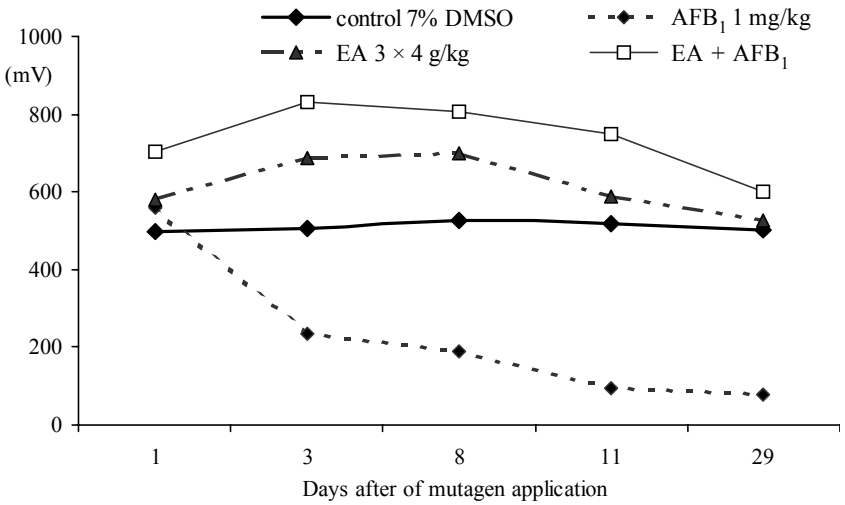

Fig. 1. Chemiluminescence test – ellagic acid + AFB_1

Table 5. Chemiluminescence test – values of chemiluminescence ± standard deviation

Day	Control[1]	AFB_1[2]	EA[3]	EA+AFB_1[4]
1st	500 ± 5.0	560 ± 7.0	580 ± 11.0	702 ± 9.5
3rd	505 ± 7.5	235 ± 6.0	688 ± 8.5	830 ± 6.0
8th	525 ± 5.5	180 ± 5.0	700 ± 6.0	806 ± 5.0
11th	520 ± 5.0	93 ± 2.5	590 ± 9.0	750 ± 7.5
29th	505 ± 9.0	80 ± 2.5	525 ± 6.0	600 ± 11.0

[1]control (7% DMSO); [2]AFB_1 (1 mg/kg); [3]ellagic acid (3 × 4g/kg); [4]AFB_1 + ellagic acid (1 mg/kg + 3 × 4g/kg)

Table 6. Chemiluminescence test – values of chemiluminescence ± standard deviation

Day	Control[1]	IQ[2]	EA[3]	EA + IQ[4]
1st	450 ± 2.5	465 ± 5.0	573 ± 7.5	868 ± 2.5
5th	438 ± 2.5	320 ± 2.5	750 ± 9.5	849 ± 5.0
7th	440 ± 5.0	260 ± 5.0	707 ± 8.5	800 ± 7.5
12th	452 ± 2.5	180 ± 7.5	650 ± 10.0	690 ± 11.0
25th	464 ± 5.5	150 ± 7.0	501 ± 9.0	524 ± 5.0

[1]control (7% DMSO); [2]IQ (20 mg/kg); [3]ellagic acid (3 × 4g/kg); [4]IQ + ellagic acid (20 mg/kg + 3 × 4g/kg)

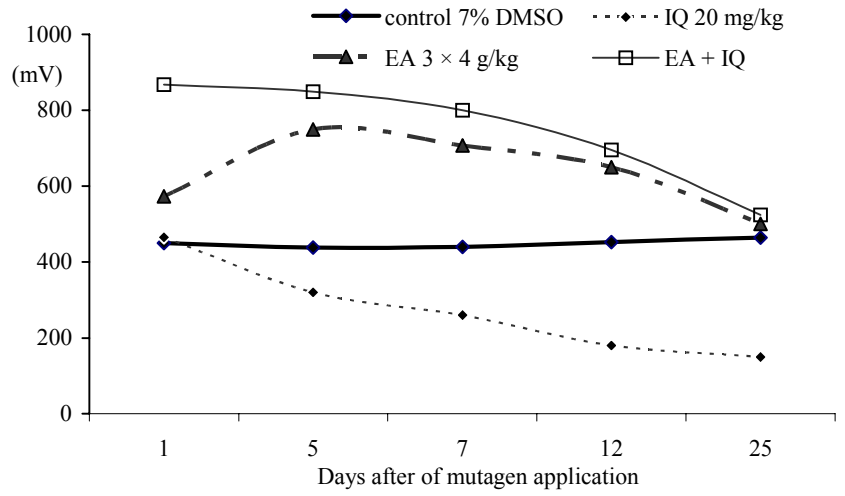

Fig. 2. Chemiluminescence test – ellagic acid + IQ

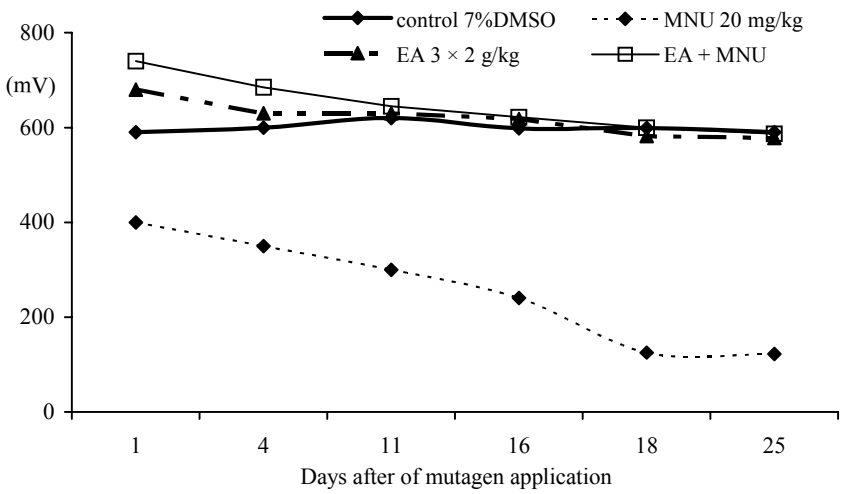

Fig. 3. Chemiluminescence test – ellagic acid + MNU

Table 7. Chemiluminescence test – values of chemiluminescence ± standard deviation

Day	Control[1]	MNU[2]	EA[3]	EA + MNU[4]
1st	590 ± 2.5	400 ± 5.0	680 ± 5.0	740 ± 7.0
4th	600 ± 5.0	350 ± 5.0	630 ± 8.5	685 ± 5.5
11th	620 ± 5.0	300 ± 5.0	630 ± 5.0	645 ± 7.5
16th	598 ± 5.0	240 ± 2.5	618 ± 5.0	622 ± 8.5
18th	599 ± 2.5	125 ± 9.5	582 ± 6.0	600 ± 5.5
25th	590 ± 1.4	122 ± 5.5	578 ± 5.5	587 ± 5.0

[1]control (7% DMSO), [2]MNU (20 mg/kg), [3]ellagic acid (3 × 2 g/kg), [4]MNU + ellagic acid (20 mg/kg + 3 × 2 g/kg)

As concerns the effect of the combination of ellagic acid (4 g/kg) and IQ, on treatment of mice with the strong mutagen and carcinogen IQ significantly reduced chemiluminescence values were found as compared to controls from the fifth day of investigations. Again, markedly increased chemiluminescence values were recorded in the group treated with ellagic acid in combination with IQ, and even with ellagic acid alone in comparison with the controls during 12 days from the begining of investigation ($P < 0.01$) (Fig. 2 and Table 6).

As to the effect of ellagic acid (2 g/kg) and MNU: during 25 days of investigation, in comparison with the controls, there was a marked decrease of chemiluminescence after the application of MNU to mice. Chemiluminescence values in the group of mice treated with ellagic acid alone differed significantly from the control group only on the first day of investigation ($P < 0.01$). In the group of mice treated with ellagic acid in combination with MNU, significant differences were found in comparison with the controls on the first and fourth day of investigation ($P < 0.01$). From the 11th day, the results of chemiluminescence in these groups did not differ significantly (Fig. 3 and Table 7).

DISCUSSION

There are numerous papers confirming the antimutagenic effect of ellagic acid. The inhibitory effect on mutagenicity induced by AFB_1 in the Ames test was described by MANDAL *et al.* (1987), GÓRSKI *et al.* (1991), LOARCAPINA *et al.* (1996, 1998), and SONI *et al.* (1997). However, FRANCIS *et al.* (1989) observed only a small reduction of AFB_1 mutagenicity although the mutagenicity of N-methyl-N′-nitro-N-nitrosoguanidine was reduced markedly. Similarly, the mutagenic effect of IQ was markedly reduced by the action of ellagic acid (AYRTON *et al.* 1992). EA was effective in inhibiting the mutagenicity of MNU in the reaction of double-stranded DNA with MNU (DIXIT & GOLD 1986, 1987). However, CHEN and CHUNG (2000) did not find any antimutagenic effect of ellagic acid regarding the direct mutagens 2-nitrofluorene, 4,4′-dinitro-2-biphenylamine, 1-nitropyrene, 1,3-dinitropyrene, 2-nitro-*p*-phenylenediamine, 3-nitro-*o*-phenylenediamine, and 4-nitro-*o*-phenylenediamine. Similarly, ellagic acid had no influence on the genotoxicity of 1-nitropyrene and 1,6-dinitropyrene (KUO *et al.* 1992).

The induction of micronuclei and chromosomal aberrations produced by the whole-body exposure to ionizing radiation in mice was found to be significantly inhibited by oral administration of ellagic acid at 200 µmol/kg b. w. (THRESIAMMA *et al.* 1998). A 3-day exposure to ellagic acid greatly reduced the degree of chromosome instability in papilomavirus-transformed cells (STICH *et al.* 1990).

According to our results, EA similarly inhibited the mutagenic effects of AFB_1, IQ and MNU in mice after a three-day exposure as revealed by a significant reduction of the number of micronuclei in the bone marrow in the groups of mice treated with EA and mutagen in comparison with the mice treated with mutagen only.

Ellagic acid which belongs to polyphenolic compounds has anticarcinogenic and anti-oxidative effects (DE MEIJA *et al.* 1999; MASUDA *et al.* 1999). Ellagic acid can contribute to the prevention of carcinogenesis by a more effective detoxication through an increased glutathione-S-transferase (GST) activity. Increased levels of glutathione and GST activity upon the application of ellagic acid to rodents were observed by VAN LIESHOUT *et al.* (1998) and GRAEME-SHEPARD *et al.* (2000). ANDERSON *et al.* (2001) described the possibility of enhancing the anti-teratogenic potential by polyphenols from nuts, especially by ellagic acid which effectively inhibited the oxidation of LDL.

In our experiments, ellagic acid acted as an effective antimutagen at the two highest concentrations tested, i.e. 300 µg and 30 µg per plate, as it significantly reduced the activity of IQ in both strains of *S. typhimurium* in the Ames test. The concentration of 300 µg per plate had the strongest effect in strain TA98 whereas in strain TA100, 30 µg per plate was more effective than 300 µg per plate. The concentrations of 3.0 and 0.3 µg per plate proved to be ineffective in our experiments. Also in combination with AFB_1, ellagic acid proved to be a strong antimutagen at the two highest concentrations followed. In this case, the lower of the two concentrations, i.e. 30 µg per plate, had a much greater antimutagenic effect than that of 300 µg per plate. Similar results were observed with all concentrations of the mutagen and in both strains. It was only in combination with the direct mutagen MNU that ellagic acid did not show any marked antimutagenic effects at most of the concentrations tested in strain TA100. The highest concentrations of ellagic acid reduced the mutagenic effect of MNU weakly and only in combination with two lower concentrations of MNU.

Our results of the Ames test that show a greater effect of ellagic acid on the mutagenity of indirect mutagens than on MNU are an evidence for a more pronounced effect of ellagic acid on the activation of promutagen although other mechanisms may also play a role. MNU as a direct mutagen was applied in relatively high concentrations that manifested mutagenicity in strain TA100 only, and the lower effect of ellagic acid on this mutagen may be the results of these concentrations. A somewhat greater effect of ellagic acid was observed at the lowest concentration of MNU. On the other hand, the effect of ellagic acid on mutagenity of indirect mutagens does not seem to depend on the concentration of the mutagen applied.

Aflatoxin B_1 as a strong mutagen and carcinogen has also immunosuppressive effects in several animal species. Inhibition of phagocytic activity was observed by MICHAEL *et al.* (1973) in chick cells, by RICHARD and THURSTON (1975) in rabbit macrophages, and by TUREK *et al.* (1994) in murine granulocytes. Similarly, upon the application of aminoacid pyrolysates formed during a high-temperature treatment of meat, we observed a reduced phagocytic activity in mice as measured by chemiluminescence (TUREK *et al.* 1994). Heterocyclic amines are transformed enzymatically to reactive substances and such reactions are controlled by anti-oxidants such as polyphenols, sulforafans, isothiocyanates, etc. (WEISBURGER 1999).

In the present paper, we followed the reaction of mice treated with ellagic acid and AFB_1, IQ, and MNU as measured by the chemiluminescence test which reflects the degree of phagocytosis by murine granulocytes. Upon the application of three different mutagens, we found, in agreement with findings in the literature, a marked inhibition of oxygen radical formation, namely of the complex: hydrogen peroxide – myeloperoxidase – halide factor in the case of AFB_1 and IQ, except for the first day of investigation, i.e. 24 h after the application of mutagen. In the experiment with ellagic acid in combination with AFB_1, ellagic acid alone at the dose of 4 g/kg stimulated chemiluminescence values most markedly on the third and eighth day of the experiment. This result is in agreement with the finding of the stimulation of the production of the superoxide radical by ellagic acid in murine peritoneal macrophages (KAUL & KHANJUDA 1999). Ellagic acid applied together with AFB_1 stimulated chemiluminescence to a greater degree than ellagic acid alone, namely up to 1.6 times in comparison with controls. Similar results were recorded upon the treatment of mice with ellagic acid and IQ. Ellagic acid stimulated chemiluminescence most markedly in combination with IQ up to the 12^{th} day of investigation (up to 1.9 times compared to controls). Different results were obtained upon the application of ellagic acid (2 g/kg) in combination with MNU. Ellagic acid alone stimulated the reaction of murine granulocytes on the 1^{st} day only, and ellagic acid in combination with MNU on the 1^{st} and 4^{th} day of investigation in this case.

From the results obtained it follows that ellagic acid effectively prevented the negative effects of mutagens in one of the phases of phagocytosis as measured by chemiluminescence. At the dose of 4 g/kg, ellagic acid in combination with mutagens stimulated the reaction of murine granulocytes more than ellagic acid alone. It is possible to presume that ellagic acid at the dose of 4 g/kg reacts in the presence of strong mutagens and carcinogens by an excessive increase of the formation of the complex hydrogen peroxide – myeloperoxidase – halide factor which is responsible for the lethal reaction in phagocytes. At the dose of 2 g/kg, ellagic acid alone as well as in combination with MNU had a predominantly immunomodulatory effect.

We may suppose that ellagic acid has protective effects as an antimutagenic and anticarcinogenic factor found in plant foods that can be used as a potent cancer chemopreventive agent in the diet, this possibly being caused, among others, by a favourable influence on immune processes.

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Souhrn

ŠMERÁK P., ŠESTÁKOVÁ H., POLÍVKOVÁ Z., BÁRTA I., TUREK B., BÁRTOVÁ J., LANGOVÁ M., ANDĚL M. (2002): **Účinky kyseliny elagové na mutagenitu a na imunitní reakci u myší**. Czech J. Food Sci., **20**: 181–191.

Pomocí Amesova testu a *in vivo* mikronukleus testu jsme sledovali antigenotoxický efekt kyseliny elagové (EA) na genotoxicitu tří mutagenů: amino-methylimidazo-chinolinu (IQ), aflatoxinu B_1 (AFB_1) a *N*-nitroso-*N*-methylurey (MNU). Kyselina elagová je fenolová sloučenina, přirozeně se vyskytující v různých druzích ovoce a zeleniny. Její efekt na imunosupresivní aktivitu mutagenů jsme rovněž sledovali *in vivo* chemiluminescenčním testem. V Amesově testu kyselina elagová v koncentracích 300 a 30 µg na misku prokazatelně inhibovala mutagenní aktivitu dvou nepřímých mutagenů IQ a AFB_1. Koncentrace 300 µg na misku měla nejsilnější efekt na mutagenitu všech koncentrací IQ v testech na kmeni TA98 *Salmonella typhimurium,* zatímco na kmeni TA100 byla koncentrace 30 mg na misku efektivnější než koncentrace 300 µg na misku. Také v kombinaci s různými koncentracemi AFB_1 se kyselina elagová chovala jako silný antimutagen. V tomto případě však nižší ze dvou účinných koncentrací – 30 µg na misku měla mnohem větší antimutagenní efekt než koncentrace 300 µg na misku na obou kmenech. V kombinaci s přímým mutagenem MNU nevykazovala kyselina elagová významný antimutagenní efekt ve většině koncentrací testovaných na kmeni TA100. Jenom nejvyšší koncentrace kyseliny elagové redukovala mutagenitu MNU slabě, a to pouze v kombinaci se dvěma nižšími koncentracemi MNU. V mikronukleus testu, třídenní p.o. aplikace kyseliny elagové předcházející aplikaci AFB_1, IQ nebo MNU

významně redukovala počty mikrojader indukované samotnými mutageny v polychromatofilních erytrocytech myší. Chemiluminescenční test na myších granulocytech prokázal, že kyselina elagová nejen zabraňuje inhibičnímu účinku mutagenů na tvorbu volných kyslíkových radikálů a peroxidu vodíku, ale jejich tvorbu v kombinaci s mutageny stimuluje dokonce ve vyšším rozsahu, než je stimulace samotnou kyselinou elagovou. Z těchto výsledků vyvozujeme, že kyselina elagová silně reparuje imunosupresivní efekt testovaných mutagenů.

Klíčová slova: kyselina elagová; amino-methylimidazo-chinolin; aflatoxin B_1; *N*-nitroso-*N*-methylurea; antimutagenní aktivita; chemiluminescence

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