Antimutagenic Effect of Curcumin and Its Effect on the Immune Response in Mice

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Abstract

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A wide array of antioxidative and anti-inflammatory substances derived from edible plants have been reported to possess chemopreventive and chemoprotective activities. Among the most extensively investigated and well-defined dietary chemopreventives is curcumin. Using the Ames test and *in vivo* micronucleus test, chemiluminescence test, blastic transformation test, and comet assay, we examined the antimutagenic effects of the chemically identified chemoprotective substance curcumin (diferuloylmethane) in the pure form on mutagenicity induced by three reference mutagens: aflatoxin B_1 (AFB_1), 2-amino-3-metylimidazo[4,5-f]quinoline (IQ), and *N*-nitroso-*N*-metylurea (MNU), and the effect of curcumin on the immunosuppression caused by these mutagens. Curcumin in the pure form showed a clear antimutagenic and immunomodulatory activities on mutagenicity and immunosuppression induced by reference mutagens.

Keywords: curcumin; antimutagenic effects; response of immune; Ames test; micronucleus test; comet assay; chemiluminescence; blastic transformation tests

It has been known that most human cancers are induced by environmental factors including chemical, radioactive, and biological factors that exist in our life environment. Epidemiological studies have indicated significant differences in the incidence of cancers among ethnic groups who have different lifestyles and have been exposed to different environmental factors. It has been estimated that some human cancers could be prevented by the modification of lifestyle including the dietary modification (Surh 2003).

The use of natural chemicals allowing suppression, retardation, or inversion of carcinogenic process is a promising approach, especially for

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the prevention of tumours. According to a large number of epidemiological studies, a high consumption of fruits and vegetables is consistently associated with a low incidence of most human cancers (Dorai & Aggarwal 2004). The dietary components as chemopreventive agents have received much attention in the public and the medical community.

Curcuma longa L., which belongs to the *Zingiberaceae* family, is a perennial herb that grows up to 1 m of height with a short stem, and is distributed throughout the tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China. As a powder, called turmeric, has been used in Asian cookery for thousands of years. Current traditional Indian medicine claims the use of its powder against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism, and sinusitis.

Curcumin – diferuloylmethane, an aromatic yellow compound isolated from the root of *Curcuma longa*, is a chemopreventive agent with a multiple mechanism of action (Surh 2002; Duvoix *et al.* 2004). It has anti-inflamatory and anti-oxidative effects (Surh *et al.* 2001), reduces lipid peroxidation, thus affecting the risk of cardiovascular diseases. Many authors proved its anticarcinogenic effects on the inhibition of tumour formation and tumour promotion of tumours induced chemically or by radiation in laboratory animals (Inano & Onoda 2002); this effect was also proved *in vitro* on human carcinoma cell lines (Ellatar & Virji 2000).

The presumption of the anti-carcinogenic effect of curcumin is also supported by the findings demonstrating the increased activation of macrophages and NK cells and the modulation of the glutathione detoxification mechanism (Bhaumik *et al.* 2000).

Curcumin was described as a good anti-angiogenesis agent in the prevention of tumour promotion and was able to inhibit human telomerase activity in MCF-7 cells (Ramachandran *et al.* 2002). The ability of curcumin to support apoptosis in cancer cells without cytotoxic effects on healthy cells is also important and was described in different cell lines (Tourkina *et al.* 2004), but in same pathologies the effect of curcumin was negative because it reduced the effect of chemotherapy on the induction of apoptosis in breast cancer cells (Somasundaram *et al.* 1997, 2002). A very important role of curcumin is in altering the metabolic activation or detoxification of mutagens and carcinogens. It inhibits cytochrome P450 enzymes involved in the activation of mutagens and carcinogens (Duvoix *et al.* 2004). There is some evidence on the induction effect of curcumin on phase II metabolising enzymes – the dietary supplementation of curcumin enhances antioxidant and phase II metabolising enzymes in ddY male mice (Igbal *et al.* 2003), but the inhibition of GST activity by high doses of curcumin was also described (Piper *et al.* 1998).

Its antioxidative effect was proven in many different systems (Betancor-Fernandez *et al.* 2003). Curcumin exerts a variety of immunomodulatory effects (Churchil *et al.* 2000). Curcuminoids have been shown to be free radical scavengers that suppress the production of superoxide by macrophages, and a significant increase of macrophage phagocytic activity was also observed in curcumin treated animals. These results indicate the immunostimulatory activity of curcumin (Lukita-Atmadja *et al.* 2002).

Given its pleiotropic effects, curcumin represents an important factor for chemoprevention of human tumours and is under great interest of research studies. In our study, we combined the methods of studying the antimutagenic effects of chemopreventive agents with the methods of testing their effects on the immune response.

The effect of curcumin as a chemically identified chemoprotective substance in the pure form on the mutagenicity or immunosuppression caused by three known mutagens AFB_1, IQ, and MNU was studied using the Ames bacterial mutagenicity test and *in vivo* micronucleus, chemiluminescence and blastic transformation tests, resp. comet assay.

In our study, we chose two different representants of mutagenes which commonly occur in the human diet – mycotoxin AFB_1, food mutagen IQ (promutagens) and, third, MNU, a direct mutagen of endogenous origin.

Aflatoxin B_1 (AFB_1) – contaminating foodstuffs 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 exerts mutagenic activity in all prokaryotic and eukaryotic testing systems (Bárta *et al.* 1998; Šmerák *et al.* 2001 and others).

The another reference mutagen, 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), one of the toxic substances contaminating foods, is formed by heat processing of foodstuffs. It is one of the most serious amino acid pyrolysates (heterocyclic amines)

with mutagenic, carcinogenic, and immunosuppressive activity (Loprieno *et al.* 1991).

N-nitroso-*N*-methylurea (MNU) is an important carcinogenic *N*-nitroso compound; unlike nitrosamines and AFB_1, it is a directly acting carcinogen requiring no metabolic activation (IARC 1978).

MATERIAL AND METHODS

Ames test. Antimutagenic activity of curcumin was tested by the Ames test (Maron & Ames 1983; Černá *et al*. 1989) on the auxotrophic hisbacterial strains of *Salmonella typhimurium* TA98 and TA100.

The mutagenic substances were applied in the following concentrations: AFB_1 in the concentrations of 10 μg, 1 μg and 0.1 μg per plate with both strains, TA98 and TA100, IQ in the concentrations of 0.1 μg, 0.01 μg and 0.001 μg per plate with the strain TA98, and in the concentrations of 10 μg, 1 μg and 0.1 μg with the strain TA100, MNU in the concentrations of 1000 μg, 100 μg and 10 μg with the strain TA100 only as these concentrations had no effect on the strain TA98. Each concentration of each mutagen was combined with four different concentrations of the antimutagen (300 μg, 30 μg, 3 μg and 0.3 μg of curcumin per plate). For the metabolic activation of the indirect mutagens (AFB_1 and IQ), the S9 fraction was used of liver homogenate from laboratory rats induced by a mixture of polychlorinated biphenyls Delor 103.

Each combination of mutagen and antimutagen was tested in two separate experiments with three plates in each experiment. Percentage of the inhibition of mutagenicity was calculated as [(No of revertants of mutagen – No of revertants of mixture of mutagen and curcumin)/No of revertants of mutagen] × 100. For the statistical analysis Student's *t*-test was used.

Experimental animals. All *in vivo* experiments (bone marrow micronucleus test, chemiluminescence test, blastic transformation method and comet assay) were carried out on ten-week-old male Balb C mice, each weighing 22–26 g (purchased from BIOTEST, Konárovice, CZ). The animals were housed under controlled light regime of 12/12 h, temperature of 20 ± 2°C, relative humidity of 60 ± 10%, and complete air recirculation 10–14-times per hour. The animals were supplied with water *ad libitum* and were fed with a commercial granulated mixture for laboratory rodents. For each group 7–10 mice were used.

Preparation of substance tested

Curcumin was applied to mice per os for three days in succession by gavage. The reference mutagens were applied once on the third day, 1–1.5 h after the application of antimutagens, in the same way.

In all 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.

In the experiments testing the antimutagenic effect, the following concentrations of curcumin and its combinations with mutagens were used.

Micronucleus test. Curcumin (Sigma-Aldrich) was applied in the dose of 5 g/kg and 1 g/kg of the murine body weight in the combinations of curcumin and aflatoxin B_1. In the combinations of curcumin and IQ or MNU, the dose was 1 g/kg b.w. in 0.1 ml/10 g of the murine body weight.

Aflatoxin B_1 (AFB_1) (Alexis Corporation) was applied to mice in the dose of 5 mg/kg b.w.

2-amino-3-methylimidazo[4,5-f]quinoline (IQ), (ICN) in the dose of 20 mg/kg b.w.

N-nitroso-*N*-methylurea (MNU), (Sigma-Aldrich) in the dose of 50 mg/kg b.w.

Comet assay. Curcumin was applied in the dose of 1 g/kg of the murine body weight in 0.1 ml/10 g of the murine body weight.

Aflatoxin B_1 (AFB_1) (Alexis Corporation) was applied to mice in the dose of 5 mg/kg b.w.

2-amino-3-methylimidazo[4,5-f]quinoline (IQ), (ICN) in the dose of 20 mg/kg b.w.

N-nitroso-*N*-methylurea (MNU), (Sigma-Aldrich) in the dose of 50 mg/kg b.w.

Chemiluminescence test and blastic transformation method. Curcumin was applied in the dose of 1 g/kg of the murine body weight in 0.1 ml/10 g of the murine body weight.

Aflatoxin B_1 was applied to mice in the dose of 1 mg/kg b.w.

2-amino-3-methylimidazo[4,5-f]quinoline (IQ), (ICN) at the dose of 20 mg/kg b.w.

N-nitroso-*N*-methylurea (MNU) (Sigma-Aldrich) at the dose of 20 mg/kg b.w.

The micronucleus test

The mouse bone marrow micronucleus test was carried out according to Schmid (1975). A total of 1000 polychromatophilic erythrocytes were scored per animal for the evaluation of the frequencies of

micronucleated polychromatophilic erythrocytes. Polychromatophilic erythrocytes were observed 24 h after the aplication of the reference mutagens to the animals pre-treated with curcumin. Each experiment was run three times. For the statistical analysis Student's *t*-test was used.

Single strand breaks – Comet assay

The alkaline version of the comet assay according to Singh *et al.* (1988) modified by Collins *et al.* (1997) was used. Briefly, the cells were embedded in agarose on a microscope slide, lysed with nonionic detergent and left to unwind the DNA in alkaline electrophoresis solution (0.3M NaOH, 10mM EDTA) for 40 min. The electrophoresis (25 V, 300 mA, 4°) results in the attraction of DNA to the anode. However, if the DNA is intact, the supercoiling prevents any significant movement. The relative amount of DNA in the tail of the comet formed reflects the number of breaks in the DNA. The per cent of DNA in the tail was converted to the number of SSB/10^9 Da using the calibration of the method by X-ray irradiation (Collins *et al.* 1996). The statistical significance of the difference between the treated and the control animals was tested by Mann-Whitney *U* test.

Chemiluminescence test

The chemiluminescence test was performed according to the modification of Šestáková *et al.* (1997). In the chemiluminescence test, the degree is determined to which phagocytes are capable of liquidating the ingested material. Well known are the mechanisms in which hydrogen peroxide participates in killing (Klebanoff 1980). H_2O_2 is synthesised in phagocytes upon receiving a signal by their membrane. The finding of a higher or lower activity of the complex H_2O_2-MPO-Cl^- (I^-) speaks of the readiness of the first defense line against bacteria, tumour cells, as well as carcinogen-altered cells. The results are presented in maximum values (mV) of the chemiluminescence

Table 1. Effect of curcumin on mutagenicity of AFB_1 – Ames test – TA98, TA100

AFB_1 + curcumin dose (μg/plate)	*S. typhimurium* TA98 + S9		*S. typhimurium* TA100 + S9	
	revertants ± SD	% inhibition	revertants ± SD	% inhibition
10 + 0	836 ± 130		1147 ± 107	
10 + 0.3	859 ± 159	+3	1128 ± 88	–2
10 + 3	846 ± 138	+1	1208 ± 71	+5
10 + 30	444 ± 146**	–47	945 ± 14*	–18
10 + 300	46 ± 9**	–95	146 ± 6**	–87
1 + 0	299 ± 125		1117 ± 161	
1 + 0.3	215 ± 28	–28	1065 ± 157	–5
1 + 3	203 ± 26	–32	1061 ± 169	–5
1 + 30	72 ± 7**	–74	551 ± 166**	–51
1 + 300	19 ± 3**	–94	61 ± 7**	–95
0.1 + 0	70 ± 16		477 ± 148	
0.1 + 0.3	72 ± 18	+3	464 ± 169	–3
0.1 + 3	74 ± 16	+6	312 ± 95	–35
0.1 + 30	37 ± 11**	–47	143 ± 32**	–70
0.1 + 300	19 ± 3**	–73	62 ± 16**	–87
Control – DMSO	21 ± 1		118 ± 9	
0 + 0.3	22 ± 2		143 ± 5	
0 + 3.0	18 ± 3		142 ± 12	
0 + 30.0	19 ± 2		101 ± 16	
0 + 300.0	18 ± 1		79 ± 6	

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

response of polymorphonuclear leucocytes in the dependence on time.

The blastic transformation method

For the study of the readiness of cells in acquired immunity, we selected a functional test assessing T-lymphocytes, the blastic transformation method (Field 1996). This test of lymphocyte activation determines the functional capacity of T-lymphocytes to react to a mitogen by proliferation, and as such it is a more direct examination of the immune competence than just determining the numbers of various lymphocyte populations (Stites & Terr 1994).

RESULTS

Ames test

Curcumin showed protective effects against mutagenicity of three different concentrations of AFB_1 (10 μg, 1 μg and 0.1 μg per plate) in both tested strains of *Salmonella typhimurium* TA98 and TA100. The effective concentrations were 30 μg and 300 μg of curcumin per plate. The inhibition of mutagenicity was dose dependent and is expressed as percentage of inhibition of mutagenic activity in Table 1. Lower concentrations 0.3 μg and 3 μg of curcumin per plate were not effective, with the exceptions of its effects on 1 μg AFB_1 per plate in TA98 and the effect of 3 μg of curcumin per plate on mutagenicity of 0.1 μg of AFB_1 per plate.

Similar strong and dose dependent inhibition effects of two highest concentrations of curcumin were detected also against mutagenicity of all concentrations of IQ (0.1 μg, 0.01 μg and 0.001 μg per plate in TA98, 10 μg, 1 μg and 0.1 μg per plate in TA100). Lower concentrations, i.e., 0.3 and 3 μg of curcumin per plate, only slightly reduced the effect of IQ, mostly not significantly (Table 2).

The effect of curcumin on the direct mutagen MNU on TA100 was less effective as two highest concentrations of curcumin reduced the muta-

Table 2. Effect of curcumin on mutagenicity of IQ – Ames test – TA98, TA100

IQ + curcumin dose (μg/plate)	*S. typhimurium* TA98 + S9		IQ + curcumin dose (μg/plate)	*S. typhimurium* TA100 + S9	
	revertants ± SD	% ihibition		revertants ± SD	% inhibition
0.1 + 0	1210 ± 99		10 + 0	1302 ± 91	
0.1 + 0.3	1109 ± 46	–8	10 + 0.3	1182 ± 132	–9
0.1 + 3	1058 ± 74*	–13	10 + 3	1186 ± 147	–9
0.1 + 30	791 ± 62**	–35	10 + 30	1169 ± 98	–10
0.1 + 300	67 ± 15**	–95	10 + 300	125 ± 26**	–90
0.01 + 0	389 ± 80		1 + 0	545 ±128	
0.01 + 0.3	350 ± 76	–10	1 + 0.3	468 ± 95	–14
0.01 + 3	329 ± 114	–15	1 + 3	362 ± 34*	–34
0.01 + 30	236 ± 69**	–39	1 + 30	263 ±45**	–52
0.01 + 300	40 ± 7**	–89	1 + 300	98 ± 5**	–82
0.001 + 0	102 ± 5		0.1 + 0	186 ± 30	
0.001 + 0.3	101 ± 11	–1	0.1 + 0.3	159 ± 19	–15
0.001 + 3	85 ± 13*	–17	0.1 + 3	157 ± 38	–16
0.001 + 30	61 ± 12**	–40	0.1 + 30	136 ± 23*	–27
0.001 + 300	28 ± 3**	–73	0.1 + 300	85 ± 12**	–54
Control – DMSO	37 ± 5			112 ± 22	
0 + 0.3	42 ± 9			144 ± 3	
0 + 3	33 ± 5			138 ± 7	
0 + 30	36 ± 1			114 ± 11	
0 + 300	26 ± 4			76 ± 4	

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

Table 3. Effect of curcumin on mutagenicity of MNU – Ames test – TA98, TA100

MNU + curcumin dose (μg/plate)	*S. typhimurium* TA100	
	revertants ± SD	% inhibition
1000 + 0	1856 ± 99	
1000 + 0.3	1849 ± 154	0
1000 + 3	1864 ± 116	0
1000 + 30	1662 ± 126*	–11
1000 + 300	1202 ± 184**	–35
100 + 0	1977 ± 83	
100 + 0.3	1877 ± 100	–5
100 + 3	1895 ± 70	–4
100 + 30	1770 ± 74**	–11
100 + 300	1536 ± 92**	–22
10 + 0	783 ± 157	
10 + 0.3	775 ± 134	–1
10 + 3	772 ± 176	–1
10 + 30	516 ± 98**	–34
10 + 300	386 ± 50**	–51
Control – DMSO	64 ± 16	
0 + 0.3	71 ± 9	
0 + 3	74 ± 13	
0 + 30	60 ± 15	
0 + 300	51 ± 17	

SD – standard deviation; *P ≤ 0.05; **P ≤ 0.01

genicity of MNU to a lower extent in comparison with the indirect mutagens. The highest effect was found in the combination of 300 μg per plate with a lower concentration of MNU – 10 μg per plate (Table 3).

Micronucleus test

In the group of animals treated with aflatoxin B_1 a statistically significant higher number was found of micronuclei in polychromatophilic erythrocytes of the bone marrow as compared with the control group. The number of micronuclei in animals influenced by curcumin alone did not differ from that of the control group. On oral application of the combination of curcumin and aflatoxin B_1 at a dose of 5 g/kg, 1 g/kg and 0.5 g/kg b.w., the number of micronuclei in polychromatophilic erythrocytes was lower in a statistically significant degree in comparison with laboratory mice treated with AFB_1 alone.

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

Substance studied	Number of micronuclei ± SD
Control – 7% DMSO	2.2** ± 0.82
Curcumin – 1 g/kg	2.6** ± 0.49
Curcumin – 5 g/kg	3.7** ± 1.3
AFB1 – 5 mg/kg	9.8* ± 2.4
Curcumin + AFB1 – 5 g/kg + 5 mg/kg	4.0** ± 1.2
Curcumin + AFB1 – 1 g/kg + 5 mg/kg	3.2** ± 1.1
IQ – 20 mg/kg	6.6* ± 1.02
Curcumin + IQ – 1 g/kg + 20 mg/kg	3.4** ± 0.80
MNU – 50 mg/kg	36.5* ± 5.85
Curcumin + MNU – 1 g/kg + 50 mg/kg	18.4** ± 5.20

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

A similar effect came about on the application of the IQ mutagen: curcumin in combination with the IQ mutagen reduced its mutagenic effect to a statistically significant degree. Likewise, on the treatment of mice with the combination of curcumin and MNU a significant reduction of the number of micronuclei in comparison with the number of micronuclei elicited by MNU alone was observed. The results are presented in Table 4.

Comet assay

The effect of curcumin on the induction of DNA damage by AFB_1, MNU or IQ was followed with bone marrow cells, liver cells, and with epithelium cells of the colon using the comet assay. In our experiment, AFB_1 did not induce any significant amount of SSB in bone marrow cells, therefore no effect of curcumin on this induction could bee seen. No induction of SSB was seen either in liver cells of animals treated with AFB_1 alone, however, some slight but significant increase of DNA breaks was observed 2 h after the application of AFB_1 to animals pre-treated with curcumin. A significant induction of the DNA damage was found in colon epithelium cells where the number of SSB increased from the value 1.33 to 2.77 SSB/10^9 Da

2 h after the aplication of AFB_1. This number of SSB decreased again to the level of the control 24 h after the application. No effect of the pretreatment of mice with curcumin was observed, either after 2 h or 24 h from the application.

In contrast to AFB_1, MNU induced DNA damage in all cell types. In bone marrow cells the level of SSB increased from the value of 0.49 SSB/10^9 Da that was found in controls to 1.7 SSB/10^9 Da. The previous application of curcumin decreased this value significantly to 1.37 SSB/10^9 Da. Twenty four hours after the applicaton of MNU, the value of SSB decreased to 0.8 SSB/10^9 Da approximately both in curcumin treated and non-treated animals. In contrast to bone marrow cells, no effect of curcumin on the induction of DNA damage by MNU was observed in liver cells. A significant number of SSB was induced in these cells within 2 h after the application of MNU (2.61 SSB/10^9 Da), which dropped to 1.2 SSB/10^9 Da after 24 hours.

Quite a high number of SSB was induced by MNU in colon epithelium cells. The number of SSB rose from the value of 1.33 SSB/10^9 Da found in the controls to 3.1 SSB/10^9 Da and this amount of SSB remained practically unchanged after 24 hours. In the mice pre-treated with curcumin, we found a significant reduction of SSB (to 2.46 SSB/10^9 Da). This amount also did not change during the 24 h interval after the treatment.

Similarly to AFB_1, IQ did not induce remarkable DNA damage in bone marrow and in liver cells. Only in liver the amount of SSB rose to 0.77 SSB/10^9 Da, which was about twice as much as the control level. However, IQ was quite efficient in inducing DNA breaks in colon epithelium cells, where we found a significant number of breaks (3.11 and

Table 5. The effect of curcumin on the DNA damage (DNA strand breaks) induced by MNU or IQ in bone marrow cells, liver cells and colon epithelium cells

Tissue	Time (h)	MNU			Curcumin + MNU			IQ			Curcumin + IQ		
		mean	SE	*P*	mean	SE	*P*	mean	SE	*P*	mean	SE	*P*
Bone marrow	2	1.70	0.06	< 0.001**	1.37	0.09	0.012§§	0.31	0.05		0.55	0.15	
	24	0.80	0.06	0.03**	0.85	0.10		0.29	0.05		0.10	0.06	
Liver	2	2.61	0.26	< 0.001**	2.57	0.07		0.77	0.04		0.56	0.11	
	24	1.20	0.17	0.02**	1.66	0.22		0.66	0.03		0.47	0.13	
Colon	2	3.12	0.11	< 0.001**	2.46	0.27	0.046§§	3.11	0.05	< 0.001**	2.26	0.50	0.036§§
	24	3.29	0.10	< 0.001**	2.43	0.24	0.04§§	2.85	0.34	< 0.001**	1.85	0.29	0.022§§

§§ the statistical significance of difference between values found in animals treated only with a mutagen and animals treated both with a mutagen and curcumin

**the statistical significance of difference between values found in control and treated animals

Table 6. The effect of curcumin on the DNA damage (DNA strand breaks) induced by AFB_1 in bone marrow cells, liver cells and colon epithelium cells

Tissue	Time (h)	Control		Curcumin		AFB_1			Curcumin + AFB_1		
		mean	SE	mean	SE	mean	SE	*P*	mean	SE	*P*
Bone marrow	2	0.49	0.08	0.43	0.05	0.34	0.06		0.53	0.12	
	24	0.45	0.04	0.46	0.09	0.40	0.05		0.18	0.06	
Liver	2	0.33	0.06	0.42	0.08	0.39	0.07		0.59	0.08	0.029**
	24	0.39	0.05	0.43	0.05	0.37	0.05		0.39	0.08	
Colon	2	1.33	0.25	1.92	0.31	2.77	0.25	0.002**	2.96	0.23	0.001**
	24	1.57	0.00	1.15	0.16	1.39	0.26		1.58	0.22	

**the statistical significance of difference between values found in control and treated animals

2.85 SSB/10^9 Da at 2 h or 24 h after treatment, respectively). These values were decreased significantly by the previous application of curcumin to 2.26 and 1.85 SSB/10^9 Da.

The results are presented in Tables 5 and 6.

Chemiluminescence test

In all the investigations, the carcinogens (AFB_1, IQ, MNU) lowered the chemiluminescence values in a statistically significant degree when compared with the control.

In the experiment with curcumin (CRC) and AFB_1, the chemiluminescence values in the CRC group of mice and the combination of CRC and AFB_1 group did not differ from the control group until the 12th day. From the 12th day of the investigation, the chemiluminescence values decreased to a statistically significant degree (day 12 $P < 0.05$; days 21–27 $P < 0.01$) only in the group of animals treated with the combination of CRC and AFB_1 in comparison with the control group of mice. Over the whole 27-day period of testing, curcumin repaired the negative (suppressive) effect of AFB_1 on the lethal phase of phagocytosis in murine granulocytes (Table 7).

In the experiment with CRC and IQ, we obtained results analogous to those in the AFB_1 experiment. The chemiluminescence values in the groups of mice treated with CRC only or with CRC and IQ did not differ from the controls up to the 12th day of follow-up. From day 20, the chemiluminescence values fell in the group of mice treated with CRC or CRC and IQ (day 20 $P < 0.05$; day 26 $P < 0.01$) as compared with the control group of mice. Curcumin repaired the suppressive effect of IQ on the function of murine granulocytes (Table 7) throughout the whole period of follow-up.

In the experiments with CRC and MNU, the chemiluminescence values in the group with CRC alone did not differ statistically to any significant degree from the values found in the control group of mice. The values in the group of mice treated with CRC and MNU, over the whole period of testing, were significantly lower (days 1, 5, and 12, $P < 0.05$; days 21 and 27, $P < 0.01$), however, over that whole period this animal group showed statistically significant higher values of chemiluminescence in comparison with the reaction of animals treated with MNU only. Curcumin significantly suppressed the negative effect of MNU on phagocytosis in murine granulocytes (Table 7).

Table 7. Chemiluminescence values after administration of curcumin and mutagens (means ± S.D.)

Day after of mutagen administration	Control (7% DMSO)	AFB1 (1 mg/kg)	Curcumin (1 g/kg)	Curcumin + AFB_1
1st	700 ± 17.2	470 ± 8.6	656 ± 11.6	740 ± 10.9
6th	697 ± 15.0	320 ± 7.2	652 ±14.4	656 ± 11.3
12th	690 ± 14.1	232 ± 9.3	644 ± 13.2	565 ± 12.1
21st	688 ± 12.3	186 ± 6.4	640 ± 11.7	541 ± 10.6
27th	691 ± 11.8	150 ± 7.8	641 ± 12.6	520 ± 12.2
		IQ 20 mg/kg	Curcumin 1 g/kg	Curcumin + IQ
1st	792 ± 13.3	506 ± 10.2	799 ± 12.1	805 ± 15.2
6th	788 ± 12.9	489 ± 9.8	810 ± 11.8	775 ± 12.0
12th	785 ± 15.2	396 ± 11.3	766 ± 14.5	723 ± 14.4
20th	790 ± 14.9	215 ± 10.8	683 ± 14.9	651 ± 13.8
26th	782 ± 15.4	192 ± 8.6	600 ± 15.0	615 ± 14.6
		MNU 20 mg/kg	Curcumin 1 g/kg	Curcumin + MNU
1st	654 ± 10.5	500 ± 7.2	632 ± 14.5	595 ± 12.6
5th	649 ± 13.5	402 ± 9.1	630 ± 12.9	580 ± 11.8
12th	646 ± 14.8	312 ± 7.5	615 ± 14.9	545 ± 15.3
21st	652 ± 15.1	222 ± 8.8	604 ± 13.4	510 ± 12.2
27th	650 ± 12.2	203 ± 5.0	589 ± 15.9	491 ± 10.5

Table 8. Blastic transformation values after administration of curcumin and mutagens (means ± S.D.)

Day after of mutagen administration	Control (7% DMSO)	AFB_1 (1 mg/kg)	Curcumin (1 g/kg)	Curcumin + AFB_1
5th	18.85 ± 3.66	4.79 ± 1.05	16.25 ± 4.0	17.05 ± 3.25
13th	22.2 ± 5.03	5.46 ± 0.72	18.88 ± 4.86	15.0 ± 2.02
19th	27.51 ± 4.5	2.97 ± 0.45	22.33 ± 3.22	18.87 ± 2.87
		IQ (20 mg/kg)	Curcumin (1 g/kg)	Curcumin + IQ
5th	20.13 ± 2.8	5.32 ± 1.32	21.45 ± 3.05	20.2 ± 3.22
13th	25.06 ± 3.12	3.98 ± 0.66	23.2 ± 2.55	19.82 ± 4.14
19th	19.88 ± 2.15	2.83 ± 0.4	17.15 ± 2.48	13.75 ± 2.25
		MNU (20 mg/kg)	Curcumin (1 g/kg)	Curcumin + MNU
5th	18.85 ± 3.2	5.14 ± 1.06	16.25 ± 2.52	19.07 ± 3.83
13th	22.2 ± 4.34	6.94 ± 1.27	18.88 ± 2.91	13.5 ± 2.54
19th	27.51 ± 4.22	4.13 ± 0.7	22.33 ± 3.74	18.77 ± 2.75

Blastic transformation method

In all the blastic transformation experiments, all the carcinogens (AFB_1, IQ, MNU) lowered the capacity of T-lymphocytes to transform into blasts in a statistically significant degree ($P < 0.01$).

In the experiment with CRC and AFB_1 over three weeks of follow-up, the stimulation indexes (SI) were found to be higher to a statistically significant degree ($P < 0.01$) in the groups of mice treated with CRC and AFB_1 in comparison with the stimulation indexes in the groups treated with AFB_1 only (Table 8). The same results were obtained in the experiment with CRC and IQ (Table 8).

In the experiment with CRC and MNU, statistically significant higher SI were found (days 5 and 19, $P < 0.01$; day 13, $P < 0.05$) in the groups of mice treated with the combination of CRC and MNU in comparison with the groups of mice treated with MNU only (Table 8).

Also in the assessment of the functional capacity of T-lymphocytes regarding blastic transformation, curcumin revealed the ability to compensate significantly the suppressive effects of all three carcinogens.

DISCUSSION

Epidemiological studies have indicated a significant difference in the incidence of cancers among ethnic groups, who have different lifestyles and have been exposed to different environmental factors (Smith-Warner *et al.* 2003). Many authors focused their attention on the study of anticarcinogenic and antimutagenic effects of phytochemicals in *in vivo* and *in vitro* tests (Anto *et al.* 2002; Park & Surh 2004).

Curcumin is an extremely potent inhibitor of mutagenicity of indirect mutagens AFB_1 and IQ in the concentration of 300 μg per plate, and a potent inhibitor in the concentration of 30 μg per plate as it was proved in Ames test with *Salmonella typhimurium* TA98 and TA100 after metabolic activation. The effect on the mutagenic activity of the direct mutagen MNU was also detected but to a much lower extent in comparison with the effect of the indirect mutagens. This is in agreement with the fact that curcumin inhibits metabolic activation of mutagens especially by inhibiting phase I enzymes (Duvoix *et al.* 2004) or affecting the detoxification enzymes (Igbal *et al.* 2003).

Similar results were achieved by several authors using the Ames test. Nagabushan *et al.* (1987) described the inhibition of mutagenicity of several indirect mutagens by curcumin but no inhibition of mutagenicity of direct mutagens sodium azide, monoacetylhydrazine, streptozocin, and 4-nitrophenylendiamine. Soni *et al.* (1997) proved a decrease of mutagenicity of AFB_1 and Shishu *et al.* (2002) the inhibition by curcumin of mutagenicity of several indirect cooked food mutagens including IQ using the Ames assay. Contrary to Nagabushan's negative results with curcumin and direct mutagens, De Flora *et al.* (1994) proved the inhibitory effect of curcumin on mutagenicity of the direct-acting mutagen 4-nitroquinoline 1-oxide (4NQO). Also in our experiments the ef-

fect of curcumin against mutagenicity of direct mutagen MNU was significant, but its effects against AFB_1 and IQ were much stronger.

In micronucleus test, we detected antimutagenic effect of curcumin against all three mutagens. Treshiama *et al*. (1998) described similar effects of curcumin on chromosomal aberrations or micronuclei induced by irradiation, and Shukla *et al*. (2002) on chromosomal aberrations induced by cyclophosphamide.

The immune system plays a significant role in the combination with prooxidative and antioxidative processes in the organism, especially in the initial stages of oncogenesis. Many substances, for example all the above mentioned mycotoxins, are important immunosupressors and play an important and critical role in the initial stages of cancer progession, because in the final stage of oncogenesis, in which it is decided whether a tumour cell shall progress or shall be eliminated, namely the controlling and liquidating action of the immune system is decisive.

Curcumin also significantly suppresses the induced oxidative stress by scavenging free radicals, and its antioxidative activity seems to be derived from its suppressive effects on the increase of the peroxisome content and the decrease of the glutathione peroxidase and D-glucose-6-phosphate dehydrogenase activities (Watanabe & Fukui 2000).

Many authors proved anticarcinogenic effects of curcumin on the inhibition of tumour formation and tumour promotion of the tumours in laboratory animals induced chemically or by radiation (Ikezaki *et al*. 2001; Inano & Onoda 2002); antiproliferative effect was also proved *in vitro* on human carcinoma cell lines (Elattar & Virji 2000). The antimutagenic effect of curcumin against various mutagens in *in vivo* and *in vitro* test systems was proved by different authors (El-Hamss *et al*. 1999; Polasa *et al*. 2004).

Curcumin blocks the tumour initiation induced by benzo(a)pyrene (B(a)P) and dimethylbenz(a)anthracene via the inhibition of the formation of DNA adducts (Huang *et al.* 1992). It is also known to inhibit skin carcinogenesis, carcinogenesis of the forestomach and colon in mice (Huang *et al.* 1994). The mechanism of anticarcinogenic effect is not fully understood. Curcumin may inhibit BaP-induced forestomach cancer in mice by affecting the activation as well as inactivation pathways of BaP metabolism in the liver (Singh *et al*. 1998). However, the induction of SSB in V79 cells *in vitro* with directly acting methylating agent MNNG was also reduced by curcumin (Chakraborty *et al*. 2004). The authors found that curcumin not only has a chemoprotective action but may also decrease the DNA damage via the stimulation of DNA repair. Besides its chemoprotective and antimutagenic effects, curcumin induces apoptosis by multiple mechanisms (Anto *et al*. 2002). Curcumin was also found to be able to induce DNA damage in human gastric mucosa cells and in isolated human lymphocytes (Blasiak *et al.* 1999).

Our results are in good agreement with those describing the antitumourigenic action of curcumin (see above). However, a significant reduction of DNA damage induced by MNU or IQ was observed only in colon epithelium cells. These cells were probably protected by curcumin present in the colon, which remained there after *per os* application in a higher concentration compared to liver or bone marrow. In the two latter organs the concentration of curcumin was probably not high enough to reduce the amount of the DNA damage induced by MNU doses used in this study.

AFB_1 and IQ induced DNA damage only in colon epithelium cells, while no significant DNA damage was found in liver or bone marrow cells. It is interesting that curcumin inhibits the DNA damage induction by IQ but it did not reduce DNA breaks induced in colon cells with AFB_1. The possibility that curcumin inhibits only some of the specific cytochromes or stimulates only certain detoxifying enzymes may not be excluded (Singh *et al*. 1998).

Functional foods and nutraceuticals constitute great promise to improve health and prevent aging-related chronic diseases (Ferrari 2004). The study of phytochemicals with chemopreventive effects and a better understanding of their health-related interactions should lead to a better use of the dietary intervention in the prevention of cancers.

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