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Short Communication

Effects of H_2O_2, Fe^{2+} and Fe^{3+} on curcumin-induced chromosomal aberrations in CHO cells

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Abstract

The effects of H_2O_2, Fe^{2+} and Fe^{3+} on curcumin-induced clastogenicity were evaluated in CHO cells. Curcumin combined with H_2O_2 did not increase the chromosomal aberrations more than expected based on a simple additive effect. In contrast, the combination of curcumin-Fe significantly decreased the total number of chromosomal aberrations and the number of abnormal metaphases. The clastogenicity of curcumin may be related to its pro-oxidant properties and its ability to generate free radicals.

Key words: CHO, chromosomal aberrations, curcumin, hydrogen peroxide, mutagenesis.

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Experiments *in vitro* and epidemiological studies have shown that some compounds present in the diet have antimutagenic and anticarcinogenic properties (Surh and Ferguson, 2003). Turmeric, a spice obtained from the rhizome of *Curcuma longa* Linn (Zingiberaceae), and its active principle curcumin, have been studied for their ability to protect cells from DNA damage (Polasa *et al.*, 2004). Curcumin, widely used as a coloring for foods, has antioxidant, antimutagenic and anticarcinogenic properties (Nagabhushan and Bhide, 1992; Aggarwal *et al.*, 2003).

Curcumin reduces chromosomal aberrations in rodent bone marrow cells exposed to gamma-radiation or treated with the antitumor drug cisplatin (Abraham *et al.*, 1993; Antunes *et al.*, 2000). However, clastogenic effects of curcumin have also been reported. An increase in the frequency of chromosomal damage has been seen in mice and rats treated acutely and chronically with curcumin (Giri *et al.*, 1990; Mukhopadhyay *et al.*, 1998), and curcumin is mutagenic in cultured Chinese hamster fibroblasts (Ishidate *et al.*, 1984). At concentrations up to 10 µg/mL, curcumin is mutagenic in cultured CHO cells and, when combined with the antitumor drugs bleomycin or doxorubicin, the frequency of chromosomal aberrations is markedly increased (Araújo *et al.*, 1999; Antunes *et al.*, 1999).

The clastogenicity of curcumin in mammalian cells *in vitro* has been attributed to a pro-oxidant action of this compound (Araújo *et al.*, 1999). In agreement with this proposed mechanism, thiourea, a hydroxyl radical scavenger, significantly inhibited the increase in chromosomal aberrations in CHO cells treated with curcumin (Araújo *et al.*, 2001). Thus, hydroxyl radicals generated by curcumin could contribute to clastogenicity *in vitro*.

A combination of Fe accessibility and high production of H_2O_2 and O_2^- results in a pro-oxidant state within cells (Meneghini, 1997). The molecular structure of curcumin is susceptible to auto-oxidation in the presence of oxygen and transition metal ions (Sahu and Washington, 1992). Indeed, curcumin is a good scavenger of H_2O_2 at high concentrations, but at low concentrations it activates the Fenton reaction to increase the production of H_2O_2 (Kuchandy and Rao, 1990).

Although, there is evidence indicating that metal ions are involved in curcumin-induced mutagenicity, there is still little on this subject in the literature. There have been no studies of the effects of H_2O_2 on curcumin-induced chro-

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mosomal aberrations in CHO cells. To examine the hypothesis that the generation of free radicals is involved in curcumin-induced clastogenicity, CHO cells were treated with a pre-determined clastogenic concentration of curcumin alone or in combination with H_2O_2, Fe^{2+} or Fe^{3+}.

Curcumin (diferuloylmethane; CAS No. 458-37-7) was purchased from Sigma Chemicals Co. (St. Louis, MO). DMSO and $FeCl_3$ were purchased from Merck (Darmstadt, F.R.G.). $FeSO_4$ and H_2O_2 were obtained from Reagen (Brazil). Dulbecco's modified Eagle's medium (DMEM) and HAM-F10 were purchased from Gibco (Invitrogen Corporation, USA). Fetal calf serum (FCS) was obtained from Cultilab (Campinas, SP, Brazil). Chinese hamster ovary cells (CHO-9) were grown as monolayers at 37 °C in 25-cm^2 flasks containing HAM-F10 plus DMEM (1:1 ratio), supplemented with 10% FCS, penicillin (0.06 mg/mL) and streptomycin (0.1 mg/mL). For all experiments, exponentially growing cells were seeded at a density of 1 x 10^6/5 mL flask. Curcumin was dissolved in 0.5% DMSO. CHO cells were incubated for 14 h with H_2O_2 (1.7, 3.4 or 6.8 µg/mL), $FeCl_3$ (1.25, 2.5 or 5.0 µg/mL) or $FeSO_4$ (1.25, 2.5 or 5.0 µg/mL) in the absence or presence of curcumin.

Colcemid (0.1 µg/mL) was added to the culture medium 2 h before fixation of the cells. Each experiment was repeated three times and 300 metaphases (100 in each experiment) were analyzed per treatment to assess the frequencies of chromosomal aberrations. The mitotic index (MI) was defined as the percentage of metaphases in 3000 cells analyzed per treatment. The differences in the number of abnormal metaphases, total number of chromosomal aberrations, and mitotic indices in the absence and presence of curcumin were compared by analysis of variance (ANOVA).

The effects of H_2O_2, Fe^{2+} and Fe^{3+} on curcumin-induced chromosomal aberrations in CHO cells are shown in Tables 1 and 2. Curcumin was not cytotoxic at up to 15 µg/mL. However, as expected, there was a significant ($p < 0.05$) increase in the total number of chromosomal aberrations and in the number of abnormal metaphases after treatment with curcumin.

Curcumin readily penetrates into the cytoplasm and can accumulate in membrane structures (Joe *et al.*, 2004), primarily because of the molecule's structure, which consists of two isomers, *i.e.* the β-diketone and enol forms. The diketone form is a potent ligand for metals such as iron (Sun *et al.*, 2002). In the presence of copper or chromium, curcumin becomes a pro-oxidant and damages DNA (Ahsan and Hadi, 1998). The antioxidant/pro-oxidant action of phenolic compounds depends on factors such as metal reducing potential and chelating behavior (Decker, 1997).

Cultured CHO cells treated with different concentrations of H_2O_2 alone (Table 1) showed a significant ($p < 0.05$) increase in chromosomal aberrations at all concentrations tested. There was a concentration-dependent response in the total number of chromosomal aberrations and in the number of abnormal metaphases in cultures treated with H_2O_2. The most frequently detected aberrations were chromatid breaks followed by chromosomal breaks. At the highest H_2O_2 concentration, a significant ($p < 0.05$) decrease in the mitotic index was also observed when compared to control cultures. In combined treatments of curcumin and H_2O_2, all of the concentrations of H_2O_2 tested increased the total number of chromosomal aberrations induced by curcumin (Table 1). However, this combination did not increase the frequency of chromosomal aberrations beyond what was expected from a simple additive effect when compared to H_2O_2 and curcumin alone (H_2O_2: 144 at the highest concentration; curcumin: 68, and curcumin + H_2O_2: 225).

The influence of iron on the effects of curcumin has been investigated because of this metal's important role in

Table 1 - Effects of different concentrations of H_2O_2 on chromosomal aberrations induced by curcumin (CMN) in CHO cells.

Concentrations (µg/mL)		MI (%)	Chromosomal aberrations			Total	Abnormal cells (Mean ± SD)
H_2O_2	CMN		Gaps	B'	B"		
0	0	11.0	1	5	0	5	1.6 ± 1.0
1.7	0	10.6	2	41	8	49[a]	13.3[a] ± 2.8
3.4	0	10.3	5	101	16	117[a]	21.3[a] ± 3.2
6.8	0	7.0[a]	3	122	22	144[a]	24.6[a] ± 2.5
0	15	10.4	11	56	12	68[a]	17.0[a] ± 4.0
1.7	15	8.1	5	117	11	128[a]	29.6[a] ± 3.2
3.4	15	9.6	4	153	17	170[a]	35.6[a] ± 3.0
6.8	15	8.6	8	198	27	225[a]	46.0[a] ± 4.9

Three hundred cells per treatment were analyzed for chromosomal aberrations, and 3000 cells were scored for MI. Gaps were not included in the total number of abnormal metaphases or in the total number of chromosomal aberrations.
B' - chromatid break; B" - chromosomal break; SD - standard deviation.
[a]$p < 0.05$ for treated *vs.* control cultures.

Table 2 - Effects of different concentrations of Fe^{2+} and Fe^{3+} on chromosomal aberrations induced by curcumin (CMN) in CHO cells.

Concentrations (μg/mL)		MI (%)	Chromosomal aberrations			Total	Abnormal cells (Mean ± SD)
Fe^{2+}	CMN		Gaps	B'	B"		
0	0	11.7	1	2	1	3	1.0 ± 0.5
1.25	0	12.6	1	2	5	7	2.3 ± 0.5
2.5	0	11.7	3	4	2	6	2.0 ± 1.0
5.0	0	10.8	1	3	2	5	1.6 ± 0.6
0	15	9.2	11	51	12	63[a]	16.6[a] ± 4.0
1.25	15	8.5	16	47	8	55[a]	13.6[a] ± 4.6
2.5	15	8.2	3	21	8	29[b]	9.0[b] ± 4.0
5.0	15	9.7	2	8	0	8[b]	2.6[b] ± 2.8
Fe^{3+}	CMN						
0	0	11.6	3	3	2	5	1.6 ± 1.1
1.25	0	8.9	3	5	2	7	2.3 ± 1.0
2.5	0	10.2	2	3	0	3	1.0 ± 0.5
5.0	0	9.9	1	4	3	7	2.3 ± 1.1
0	15	7.8	12	71	7	78[a]	18.3[a] ± 6.0
1.25	15	7.1	4	35	4	39[b]	10.0[b] ± 3.5
2.5	15	10.5	0	11	4	15[b]	5.3[b] ± 2.5
5.0	15	7.8	0	9	4	13[b]	4.3[b] ± 1.5

Three hundred cells per treatment were analyzed for chromosomal aberrations, and 3000 cells were scored for MI. Gaps were not included in the total number of abnormal metaphases or in the total number of chromosomal aberrations.
B' - chromatid break; B" - chromosomal break; SD - standard deviation.
[a]$p < 0.05$ for treated vs. control cultures.
[b]$p < 0.05$ for combined treatment *vs.* curcumin alone.

biological processes such as oxygen transfer and DNA synthesis (Bernabé-Pineda *et al.*, 2004). Table 2 shows that neither Fe^{2+} nor Fe^{3+} alone was clastogenic in any of the concentrations tested. The mitotic indices were also unaffected when compared to control cultures. The combined treatments with curcumin and Fe caused a significant ($p < 0.05$), concentration-dependent decrease in the total number of chromosomal aberrations and in the number of abnormal metaphases. At 2.5 or 5.0 μg of Fe^{2+}/mL, there was a significant reduction in the total number of chromosomal aberrations induced by curcumin from 63 to 29 (54%) and 8 (87%), respectively. A similar response was seen in CHO cells treated with Fe^{3+} (Table 2).

The simultaneous addition of Fe^{2+} or Fe^{3+} significantly protected CHO cells against curcumin-induced chromosomal damage in a concentration-dependent manner compared to treatment with curcumin alone. This suggested the possibility that curcumin bound strongly to Fe. The reaction between the complexes curcumin-Fe^{2+} and curcumin-Fe^{3+} studied in aqueous media using UV spectrophotometry and cyclic voltammetry also showed a similar behavior, and indicated that a chemical reaction had occurred between the curcumin and Fe before the formation of the complexes (Bernabé-Pineda *et al.,* 2004).

Verma and Goldin (2003) recently reported that in the presence of proteins Cu^{2-} ions may not react with curcumin to generate DNA damaging species, in contrast to the data obtained with a curcumin-Cu^{2-} combination in cell-free systems (Ahsan and Hadi, 1998). These authors suggested that the inhibitory effect of Cu^{2-} ions could be partly attributed to the presence of protein in the medium since the binding of copper to proteins would influence the activity of curcumin (Verma and Goldin, 2003). In the experiments described here, the presence of serum proteins in the medium could have influenced the activity of the curcumin-Fe complex. Although the exact mechanism by which curcumin induces chromosomal aberrations remains to be elucidated, the clastogenic activity of this compound is apparently related to its pro-oxidant properties and to its ability to generate free radicals.

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