Effects of H$_2$O$_2$, Fe$^{2+}$ and Fe$^{3+}$ on curcumin-induced chromosomal aberrations in CHO cells

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Abstract

The effects of H$_2$O$_2$, Fe$^{2+}$ and Fe$^{3+}$ on curcumin-induced clastogenicity were evaluated in CHO cells. Curcumin combined with H$_2$O$_2$ 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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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$_2$O$_2$, 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$_2$O$_2$ 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$^5$/5 mL flask. Curcumin was dissolved in 0.5% DMSO. CHO cells were incubated for 14 h with H$_2$O$_2$ (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$_2$O$_2$, 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$_2$O$_2$ 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$_2$O$_2$. The most frequently detected aberrations were chromatid breaks followed by chromosomal breaks. At the highest H$_2$O$_2$ 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$_2$O$_2$, all of the concentrations of H$_2$O$_2$ tested increased the total number of chromosomal aberrations and in the number of abnormal metaphases in cultures treated with H$_2$O$_2$. The most frequently detected aberrations were chromatid breaks followed by chromosomal breaks. At the highest H$_2$O$_2$ 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$_2$O$_2$, all of the concentrations of H$_2$O$_2$ tested increased the total number of chromosomal aberrations in the absence vs. control cultures.

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>MI (%)</th>
<th>CHROMOSOMAL ABBERRATIONS</th>
<th>TOTAL</th>
<th>ABNORMAL CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
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<tr>
<td><strong>H$_2$O$_2$</strong></td>
<td><strong>CMN</strong></td>
<td><strong>Gaps</strong></td>
<td><strong>B$^+$</strong></td>
<td><strong>B$^-$</strong></td>
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<tr>
<td>0</td>
<td>0</td>
<td>11.0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1.7</td>
<td>0</td>
<td>10.6</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
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<td>10.3</td>
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<td>101</td>
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<tr>
<td>6.8</td>
<td>0</td>
<td>7.0$^*$</td>
<td>3</td>
<td>122</td>
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<tr>
<td>0</td>
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<td>11</td>
<td>56</td>
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<tr>
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<tr>
<td>3.4</td>
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<td>9.6</td>
<td>4</td>
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<tr>
<td>6.8</td>
<td>15</td>
<td>8.6</td>
<td>8</td>
<td>198</td>
</tr>
</tbody>
</table>

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.

$^*$p < 0.05 for treated vs. control cultures.
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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