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Effect of curcumin on hydroxyl radical generation through Fenton reaction

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Summary

The effect of curcumin on the production of hydroxyl radicals through the Fenton reaction has been studied. This study shows that curcumin can reduce ferric ion to ferrous and this ferrous ion can generate a hydroxyl radical in the presence of hydrogen peroxide through the Fenton reaction.

Introduction

In a recent study curcumin has been reported to catalyse the depolymerization of hyaluronic acid in vitro by oxygen-free radicals (Tonnesen, 1989). It was postulated that curcumin either influences the Haber–Weiss reaction or acts as a source of hydroxyl radical. Our earlier studies on anti-inflammatory styryl ketones showed that while some of them can act as scavengers of oxygen radicals, some can stimulate the production of oxygen radicals (Lovina and Rao, 1989; Lovina et al., 1989). Curcumin is also a potent anti-inflammatory styryl ketone. In the present study we have shown that curcumin generates a hydroxyl radical through the Fenton reaction. The ferric ion was found to get reduced by curcumin to ferrous and the ferrous ion in presence of hydrogen peroxide generates a hydroxyl radical through the Fenton reaction.

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Materials and Methods

Materials

Curcumin, *o*-phenanthroline, *p*-nitrosodimethyl aniline (NDA), 2-deoxy-D-ribose, and thiobarbituric acid were from Sigma Chemical Co. All other chemicals were of analytical grade.

Curcumin (5 mg) was dissolved in 5 ml cold NaOH solution (0.1%) and immediately diluted to the required concentration with phosphate buffer, pH 7.4 (20 mM). Ferric chloride solution was made in distilled water. Both curcumin and ferric chloride solutions were prepared freshly before use.

Reduction of Fe^{3+} to Fe^{2+} by curcumin

Reduction of Fe^{3+} to Fe^{2+} was measured by *o*-phenanthroline complex method (Cohen and

Sinett, 1982). Reaction mixture consisted of *o*-phenanthroline (0.5 mg), ferric chloride (0.1 mM), EDTA (0.1 mM) and curcumin in the final volume of 2 ml phosphate buffer, pH 7.4 (20 mM). After incubating for 10 min at ambient temperature, the absorbance was measured at 510 nm. In another experiment sodium dithionate (0.3 mM) was added instead of curcumin and the absorbance obtained was taken as an equivalent to 0.1 mM Fe^{2+} ion. To find the optimum time required for reduction, the absorbance of the reaction mixture containing 85 μM curcumin was measured for 10 min.

Production of hydroxyl radical. Measurement by NDA bleaching method

Bleaching of NDA is a sensitive method for detecting and measuring hydroxyl radical (Singh, 1982). To the reaction mixture containing NDA (6 μM), ferric chloride (0.1 mM), EDTA (0.1 mM), hydrogen peroxide (2 mM) in phosphate buffer, pH 7.4 (20 mM) were added various concentrations of curcumin to give a final volume of 2.8 ml. Absorbance was measured at 440 nm. The percentage of bleaching was calculated from the control where no curcumin was present. Experiments were done in triplicate.

Production of hydroxyl radical. Measurement by degradation of deoxyribose method

Hydroxyl radical can also be measured by the deoxyribose method (Halliwell et al., 1987). In the present experiment curcumin has been added in place of ascorbic acid. In the original method ascorbic acid was used to reduce ferric ions. To the reaction mixture containing deoxyribose (3 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), hydrogen peroxide (2 mM) in phosphate buffer, pH 7.8 (20 mM) were added various concentrations of curcumin to give a final volume of 3 ml. After incubation for 30 min at ambient temperature, trichloroacetic acid (0.5 ml, 5%) and thiobarbituric acid (0.5 ml, 1%) was added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was measured at 560 nm.

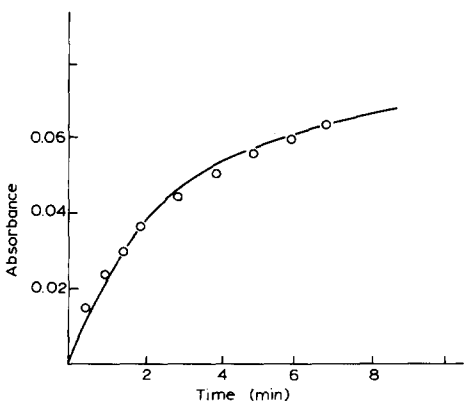

Fig. 1. Reduction of Fe^{3+} to Fe^{2+} by curcumin measured as increase in absorbance of phenanthroline complex at 510 nm.

TABLE 1

Reduction of Fe^{3+} to Fe^{2+} by curcumin

Curcumin (μM)	Fe^{2+} ion generated (mM)
1.4	0.0064
2.7	0.0107
14.0	0.0143
27.0	0.0192
140.0	0.0368

Results and Discussion

Figure 1 gives the rate of reduction of Fe^{3+} by curcumin. The Fe^{2+} generated combines with phenanthroline to give the chromogen. The absorbance increases rapidly up to 5 min and the reaction is almost complete by 10 min. Increase in the concentration of curcumin results in increase in the concentration of ferrous ion (Table 1).

TABLE 2

Generation of hydroxyl radical by curcumin measured by p-nitroso dimethyl aniline (NDA) bleaching

Curcumin (μM)	Bleaching of NDA (%)
0.96	15.1
4.80	22.8
9.60	33.1
48.00	59.2
96.40	64.6

TABLE 3

Generation of hydroxyl radicals by curcumin, measured by deoxyribose method

Curcumin (μM)	Absorbance A650
9.0	0.011
14.6	0.040
90.0	0.051
146.0	0.075

Table 2 shows the bleaching of NDA by curcumin. There is a dose-dependent increase in bleaching. Bleaching of NDA is a sensitive method for the detection of a hydroxyl radical. The bleaching is specific to the hydroxyl radical since other oxygen radicals like superoxide, singlet oxygen do not bleach NDA (Singh, 1982). To further test the specificity, mannitol was added to the reaction mixture. Mannitol inhibited the bleaching of NDA in a dose-dependent manner. Mannitol is a specific scavenger of the hydroxyl radical. These results show that the hydroxyl radical is generated in the presence of curcumin. Similar results are obtained by the deoxyribose method (Table 3). Increase in curcumin concentration results in increase in the chromogen formation. On exposure to the hydroxyl radical deoxyribose is degraded. The degraded products, on heating under acidic condition, give malonaldehyde which is detected by its ability to react with thiobarbituric acid to give a pink chromogen (Halliwell et al., 1987). The amount of chromogen formed is directly proportional to the amount of hydroxyl radical generated. Thus the data in Table 3 show that increased concentration of curcumin results in increased hydroxyl radical formation.

The Fenton reaction has been proposed as one of the important reactions for the generation of hydroxyl radicals (Halliwell and Gutteridge, 1984).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

In the Fenton reaction, ferrous ion reacts with hydrogen peroxide to generate the hydroxyl radical ($OH^{\cdot}$). During the reaction ferrous is oxidized to ferric. In the presence of a reducing agent ferrous ions can be regenerated which results in more hydroxyl radical generation. In the body, superoxide is known to reduce ferric ions as a part of the Haber–Weiss reaction to generate hydroxyl radicals. Other substances like ascorbic acid can also reduce ferric ions (Halliwell and Gutteridge, 1984). In the present study generation of the hydroxyl radical in Tables 2 and 3 can be attributed to the Fenton reaction. Addition of curcumin results in the reduction of ferric ions. The ferrous ions formed react with hydrogen peroxide to generate hydroxyl radicals.

The present study also shows that curcumin, being an anti-inflammatory agent, still promotes the production of a hydroxyl radical which itself is an inflammatory mediator. Similar observations have been made earlier. Indomethacin a potent anti-inflammatory agent was found to stimulate oxygen radicals from human neutrophils (Dale and Penfield, 1987). One of the styryl ketones which showed good anti-inflammatory activity also stimulated the production of superoxide (Lovina and Rao, 1989). In conclusion the present study shows that curcumin can reduce ferric ions to promote the Fenton type reaction to generate a hydroxyl radical in the presence of hydrogen peroxide.

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