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Curcumin inhibits iron-dependent lipid peroxidation

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Summary

Curcumin inhibited lipid peroxidation induced by ferric ions, ferrous ions and ferric-ADP chelate (in the presence of ascorbic acid or NADPH) in rat brain homogenate and liver microsomes. This may represent one of the mechanisms through which curcumin exhibits anti-inflammatory and anticancer activities.

Introduction

Lipid peroxidation is an important pathophysiological event in a variety of diseases, drug toxicities and ischemic injuries (Gutteridge and Halliwell, 1990). The involvement of iron in lipid peroxidation is well recognised, although, perhaps not clearly understood. Both ferric and ferrous ions or their chelates can precipitate the formation of oxygen radicals and may either initiate or take part in lipid peroxidation (Minotti and Aust, 1987). Formation of hydroxyl radical (Gutteridge et al., 1979) or decomposition of lipid hydroperoxides (Braughler et al., 1987), both catalysed by iron, has been implicated in lipid peroxidation. In many cases, formation of perferyl or ferryl iron species (Koppenol and Liebman, 1984) or the absolute ratio of ferric to ferrous (Braughler et al., 1986) has been suggested as important for lipid peroxidation. Regardless of the mechanism, a central role for iron in lipid peroxidation is widely accepted.

Curcumin (diferuloyl methane), from *Curcuma longa* has many interesting pharmacological activities including anti-inflammatory and anticancer activities (Srimal, 1987; Ammon and Wahl, 1991). We have conducted several studies aimed at the elucidation of its mechanism of action (Kunchandy and Rao, 1989, 1990; Susan and Rao, 1991, 1992; Unnikrishnan and Rao, 1992). The above investigations have shown that curcumin is a good antioxidant. The antioxidant properties of curcumin have also been studied by others with reference to its ability to inhibit lipid peroxidation. Sharma (1976) reported the inhibition of auto-oxidation of lipids in brain homogenates. Further, it was shown that curcumin inhibits carrageenan-induced lipid peroxidation in liver homogenates of oedemic mice (Sharma et al., 1972). However, curcumin has not been investigated, so far, for its ability to inhibit iron-catalysed lipid

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peroxidation, although such study is more relevant physiologically. In the present study, we have investigated the effect of curcumin on lipid peroxidation induced by ferric and ferrous ions. Further, we have also studied lipid peroxidation induced by ferric-ADP chelate in the presence of physiologically relevant reducing agents like ascorbic acid or NADPH.

Materials and Methods

Curcumin

None of the commercial curcumin samples is pure; all contain small amounts of desmethoxycurcumin and bisdemethoxycurcumin. Hence, pure curcumin was synthesised by condensing vanillin with acetylacetone as a boron complex according to the method of Pabon (1967). The purity and chemical structure were confirmed by NMR, mass spectroscopy, IR and elemental analysis.

Preparation of rat brain homogenates

Rat brain homogenate 10% (w/v) was prepared in 0.15 M KCl and centrifuged at $800 \times g$ for 10 min. The supernatant was used for the study of in vitro lipid peroxidation (Sharma, 1976).

Preparation of rat liver microsomes

Rat liver microsomes were prepared by sedimenting the $10\,000 \times g$ supernatant of a 0.25 M sucrose homogenate of rat liver at $105\,000 \times g$ for 60 min. The surface of the tightly packed microsomal pellet was thoroughly rinsed with 0.15 M KCl to remove the bulk of adhering sucrose, which interferes with malonaldehyde determination (Ernster and Nordenbrand, 1967). The protein content was determined by the method of Lowry et al. (1951).

Lipid peroxidation in brain homogenate

Fe^{2+}/Fe^{3+} incubations The incubation mixture contained in a final volume of 1.5 ml, brain homogenate (0.5 ml, 10% w/v), KCl (0.15 M) and ethanol (10 μl) or curcumin dissolved in ethanol. Peroxidation was initiated by adding, to the given final concentration stated, ferrous sulphate (100 μM) or ferric chloride (100 μM). After incubation for 20 min at 37°C, the reaction was stopped by adding 2 ml of ice-cold 0.25 N HCl containing 15% trichloroacetic acid, 0.38% thiobarbituric acid, and 0.05% butylated hydroxytoluene (BHT). Following heating at 80°C for 15 min, samples were cooled, centrifuged at $1000 \times g$ for 10 min and the absorbance of the supernatant measured at 532 nm. The amount of lipid peroxidation was determined using a molar extinction coefficient of 1.56×10^5 and expressed as thiobarbituric acid-reactive substances (TBARS) (Braughler et al., 1986). Control experiments without curcumin were conducted in an identical manner. A correction was made in both test and control, for spontaneous peroxidation by conducting experiments in the absence of inducing agents. Results are expressed as percent TBARS inhibition (mean $\pm$ S.E.; $n = 3$).

Iron-ADP ascorbate incubations Lipid peroxidation is also induced by iron-ADP complex in the presence of ascorbic acid (Sugioka, 1987). Iron-ADP chelate promotes redistribution of iron from water into the lipid phase and also helps to keep the iron in solution, thereby rendering it redox active (Halliwell and Gutteridge, 1990). The incubation mixture contained 1.5 ml rat brain homogenate (10% w/v) with or without curcumin at a variety of concentrations and 100 μM ferric-1.7 mM ADP-500 μM ascorbate. Peroxidation was initiated by the addition of ascorbate. TBARS was estimated as above.

Lipid peroxidation in rat liver microsomes

Fe^{2+}/Fe^{3+} incubations The peroxidation of liver microsomes (0.4 mg microsomal protein) was studied in a similar manner for ferrous and ferric stimulation.

Fe^{3+}-ADP-NADPH incubation Enzymatic lipid peroxidation in the microsomes was studied by using ferric-ADP complex and NADPH. This system makes use of cytochrome P450 as the ultimate reductant (Minotti, 1992). The incubation mixture contained microsomes (0.4 mg protein). Peroxidation was induced by adding ferric chloride (100 μM)-ADP (1.7 mM) and NADPH (400 μM) (Wiseman et al., 1990).

Iron solutions were prepared in distilled water and other solutions in 0.15 M KCl. Since most buffers trap hydroxyl radical or interfere with iron (Braughler et al., 1986), the reactions were unbuffered and carried out in 0.15 M KCl. All solutions were prepared fresh before use.

Results

Lipid peroxidation in rat brain homogenates

Compared to various stimulating agents, ferric ions were most effective in inducing lipid peroxidation. In the presence of 100 μM ferric ions, the amount of TBARS formed was 98.3 nmol/ml of the homogenate. The corresponding amounts, in the case of ferrous ion and ferric-ADP-ascorbate stimulation, were 19.9 and 85.5 nmol/ml of the homogenate. These values were obtained in the presence of BHT which prevents the additional formation of TBARS during heating due to breakdown of lipid hydroperoxides (Braughler et al., 1986). The effect of curcumin on iron stimulated lipid peroxidation is given in Table 1. In all the cases curcumin showed concentration-dependent inhibition of lipid peroxidation. At lower concentrations, curcumin was less effective in inhibiting ferrous stimulated lipid peroxidation compared to ferric and ferric-ADP-ascorbate systems. However, at concentrations above 10 μM, there was almost complete inhibition in all the cases.

TABLE 1

Effect of curcumin on the lipid peroxidation stimulated by iron and iron chelate in rat brain homogenate

[Curcumin] (μM)	Inhibition of TBARS (%) [a]		
	Fe^{2+}	Fe^{3+}	Fe^{3+}-ADP-ascorbic acid
0.25	3.2±1.0	9.4±0.9	10.0±0.9
0.5	26.4±1.4	39.1±1.1	40.3±1.0
1.0	39.3±1.3	60.9±1.8	63.7±1.5
5.0	76.1±1.9	83.2±1.7	85.4±1.7
10.0	93.5±2.0	96.4±1.6	96.9±2.0
20.0	100.0	100.0	100.0

[a] Reaction was initiated by rapid addition of iron stimulants to incubation mixture containing brain homogenates (0.5 ml, 10% w/v), curcumin and KCl (0.15 M) to a final volume of 1.5 ml. The reaction was stopped after 20 min and TBARS formed was measured in the presence of 0.05% BHT. The final concentrations of the stimulants added were: ferrous (100 μM) or ferric 100 μM of ferric (100 μM)-ADP (1.7 mM)-NADPH (100 μM), respectively. Percent inhibition was calculated by comparison with control experiments without curcumin and expressed as mean ± S.E. ($n = 3$).

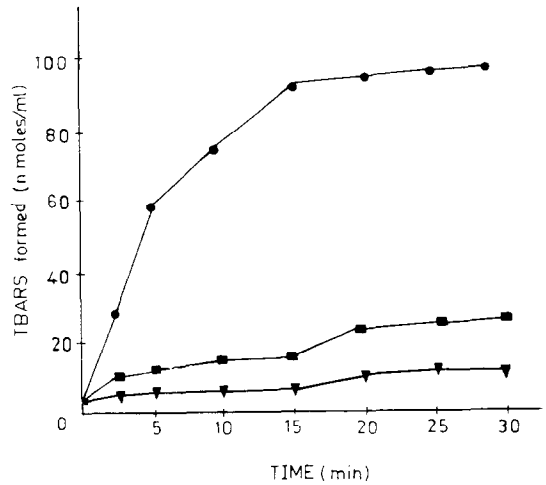

Fig. 1. Effect of curcumin on the rate of iron-stimulated lipid peroxidation in rat brain homogenate. Fe^{3+} (final concentration (100 μM) was added to the incubation mixture containing brain homogenate (0.5 ml, 10% w/v), curcumin (10 μM) and KCl (0.15 M) in a final volume of 1.5 ml. TBARS formed was measured at different intervals of time. (▼——▼) Without ferric; (●——●) with ferric, without curcumin; (■——■) with ferric, with curcumin.

Fig. 1 shows the effect of curcumin on the rate of lipid peroxidation stimulated by ferric ions. In the presence of curcumin there was a rapid decrease in the rate of TBARS formation. The effect was visible during the early stage itself. Similar results were obtained with ferrous ions and ferric-ADP-ascorbate stimulated lipid peroxidation (data not shown).

Lipid peroxidation in rat liver microsomes

In the case of rat liver microsomes as well, ferric ions stimulated the greatest amount of lipid peroxidation. In the presence of 100 μM ferric ions, the amount of TBARS formed was 108.8 nmol/mg microsomal protein. The ferrous ion and ferric-ADP-NADPH stimulation amounted to 34.2 and 89.1 nmol/mg protein, respectively. Here also, curcumin exhibited concentration-de-

TABLE 2

Effect of curcumin on lipid peroxidation stimulated by iron and iron chelate in rat liver microsome

[Curcumin] (μM)	Inhibition of TBARS (%) [a]		
	Fe^{2+}	Fe^{3+}	Fe^{+}-ADP-NADPH
0.25	1.0 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
0.5	5.1 ± 0.9	4.3 ± 1.2	4.0 ± 1.0
1.0	13.9 ± 0.9	13.2 ± 1.3	10.4 ± 1.1
5.0	48.7 ± 1.7	44.3 ± 1.6	39.7 ± 1.4
10.0	73.2 ± 1.4	69.1 ± 1.4	60.4 ± 1.9
20.0	99.0 ± 1.7	99.1 ± 1.9	98.5 ± 2.0

[a] Reaction was initiated by rapid addition of iron stimulants to incubation mixture containing rat liver microsomes (0.4 mg protein), curcumin and KCl (0.15 M) in a final volume of 1.5 ml. Percent inhibition of TBARS formed was measured as in Table 1. The final concentrations of stimulant added were similar to those in Table 1 except that ascorbic acid was replaced by NADPH (400 μM). Percent inhibition was calculated by comparison with control experiments without curcumin and expressed as mean ± S.E. ($n = 3$).

pendent inhibition of lipid peroxidation stimulated by iron (Table 2). The extent of inhibition is almost equal in the case of both ferric and ferrous ions, whereas the inhibition was slightly lower in the case of ferric-ADP-NADPH. However, at 20 μM the inhibition was almost complete in all cases.

Discussion

The addition of iron or their chelates resulted in intense lipid peroxidation and this peroxidation was inhibited by curcumin in a dose-dependent manner. In the case of brain homogenate, the inhibition was greater in ferric ions or its chelate model than in the ferrous model. Our earlier studies (Kunchandy and Rao, 1989) and a recent investigation (Tønnesen and Greenhill, 1992) have shown that ferric ions undergo reduction in the presence of curcumin. Thus, in the present study, the total amount of ferric ions available for stimulation may be partly reduced by curcumin to ferrous ions which are less effective in stimulating lipid peroxidation. In the case of liver microsomes, similar logic fails to explain the action of curcumin which inhibits both ferrous and ferric stimulated lipid peroxidation to almost equal extent. It has been recently reported that microsomal membranes contain a nonheme iron which serves in vitro for the peroxidation of lipids (Minotti, 1992). Also, in the present study, liver microsomes were isolated through ultracentrifugation and microsomes isolated by such methods are known to become contaminated with hemosiderin and ferritin which are additional sources of iron (Minotti, 1992). Thus, although 100 μM of ferric or ferrous ion were added in the present study to stimulate lipid peroxidation, the exact concentration of iron may be different.

A number of aminosteroids have been developed as a new class of CNS agents which inhibit iron-catalysed lipid peroxidation. These agents act through iron chelation (Braughler et al., 1988). Tønnesen and Greenhill (1992) reported that curcumin forms a 1 : 1 complex with iron through its 1,3-diketone system. Besides the antioxidant properties, the ability of curcumin to chelate iron may play an important role in its inhibition of iron catalysed lipid peroxidation. Since lipid peroxidation is involved in inflammation and cancer, the ability to inhibit iron-catalysed peroxidation may represent one of the mechanisms through which curcumin exhibits anti-inflammatory and anticancer activities.

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