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Curcuminoids as Potent Inhibitors of Lipid Peroxidation

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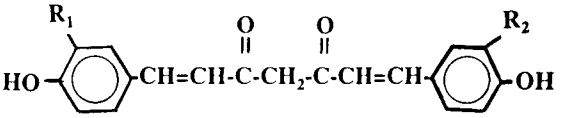
Abstract—Earlier studies showed that curcumin is a potent inhibitor of iron-catalysed lipid peroxidation. Demethoxycurcumin, bisdemethoxycurcumin and acetylcurcumin were tested for their ability to inhibit iron-stimulated lipid peroxidation in rat brain homogenate and rat liver microsomes. Comparison of the results with curcumin showed that all compounds are equally active, and more potent than α-tocopherol. These results showed that the methoxy and phenolic groups contribute little to the activity. Spectral studies showed that all compounds could interact with iron. Thus, the inhibition of iron-catalysed lipid peroxidation by curcuminoids may involve chelation of iron.

Curcumin (diferuloyl methane) from *Curcuma longa* has many interesting pharmacological effects including anti-inflammatory and anti-cancer activities (Srimal 1987; Ammon & Wahl 1991). We have conducted several studies in an effort to understand its mechanism of action (Kunchandy & Rao 1989, 1990; Susan & Rao 1991, 1992; Unnikrishnan & Rao 1992). Those studies have shown that curcumin is a good antioxidant. Our recent study has also shown that curcumin is a potent inhibitor of lipid peroxidation catalysed by iron and its chelates in rat brain homogenate and rat liver microsomes (Sreejayan & Rao 1993). The effect of curcumin on lipid peroxidation has also been studied in various models by several authors (Sharma et al 1972; Sharma 1976; Tonnesen et al 1993).



$R_1 = R_2 = OCH_3$	Curcumin
$R_1 = H, R_2 = OCH_3$	Demethoxycurcumin
$R_1 = R_2 = H$	Bisdemethoxycurcumin

FIG. 1. Structure of curcumin and related compounds.

In addition to curcumin, the rhizome of *Curcuma longa* also contains the structurally related compounds, demethoxycurcumin and bisdemethoxycurcumin (Fig. 1). Since a methoxy group adjacent to the phenolic group can provide a metal-chelating site in addition to the already present 1,3-diketone system in all three compounds, we found it interesting to study the effect of deletion of the methoxy group on iron-catalysed lipid peroxidation.

Materials and Methods

Materials

Curcumin and bisdemethoxycurcumin were synthesized as described by Pabon (1964). Demethoxycurcumin was isolated from the rhizome of *Curcuma longa* by preparative thin layer chromatography (TLC). Acetylcurcumin was prepared from curcumin as described by Roughley & Whiting (1973). The structures of all compounds were confirmed by melting point, elemental analysis (C and H) and spectral studies (IR, NMR and mass spectra).

Curcumin: mp 176°C (Lit. 177–178°C); Found: C, 68·5; H, 5·34. Calc. for $C_{21}H_{20}O_6$: C, 68·5; H, 5·45%; M^+ 368. Demethoxycurcumin: mp 170°C (Lit. 172–174°C); Found: C, 71·4; H, 5·15. Calc. for $C_{20}H_{18}O_5$: C, 71·0; H, 5·35%; M^+ 338. Bisdemethoxycurcumin: mp 225°C (Lit. 224–226°C); Found: C, 74·00; H, 5·0; Calc. for $C_{19}H_{16}O_4$: C, 74·02; H, 5·1%; M^+ 308·10. Acetylcurcumin: mp 170°C (Lit. 172–174°C); Found: C, 66·7; H, 5·55; Calc. for $C_{25}H_{24}O_8$: C, 66·35; H, 5·3%; M^+ 452.

Adenosine diphosphate (ADP), NADPH, butylated hydroxy toluene, α-tocopherol and 2-thiobarbituric acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ascorbic acid, ferrous sulphate heptahydrate and ferric chloride hexahydrate were of analytical grade.

Preparation of rat brain homogenate

Rat brain homogenate 10% (w/v) was prepared in 0·15 M KCl and centrifuged at 800 *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 *g* supernatant of a 0·25 M sucrose homogenate of rat liver at 105 000 *g* for 60 min. The surface of the tightly packed microsomal pellet was thoroughly rinsed with 0·15 M KCl to remove adhering sucrose, which interferes with malonaldialdehyde determination (Ernster & 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 the test compound dissolved in ethanol. Peroxidation was initiated by adding,

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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 ice-cold 0·25 M HCl containing 15% trichloroacetic acid, 0·38% thiobarbituric acid, and 0·05% butylated hydroxytoluene. Following heating at 80°C for 15 min, samples were cooled and centrifuged, at 1000 g for 10 min, and the absorbance of the supernatant was measured at 532 nm. The amount of lipid peroxidation was determined using the molar extinction coefficient of $1{\cdot}56 \times 10^5$ and expressed as thiobarbituric acid reactive substances (Braughler et al 1986). Control experiments without test compound 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.

Iron ADP-ascorbate incubations

Lipid peroxidation is also induced by iron-ADP complex in the presence of ascorbic acid (Sugioka et al 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 & Gutteridge 1990). The incubation mixture contained in a final volume of 1·5 mL, brain homogenate (0·5 mL, 10% w/v) with test compounds at a variety of concentrations and 100 μM Fe^{3+}, 1·7 mM ADP and 500 μM ascorbate. Peroxidation was initiated by the addition of ascorbate. Thiobarbituric acid reactive substances were estimated as described above.

Lipid peroxidation in rat liver microsomes

Fe^{2+}/Fe^{3+} incubations. The peroxidation of rat liver microsomes (0·4 mg microsomal protein) was studied in a similar manner for Fe^{2+} and Fe^{3+} stimulation.

Fe^{3+}-ADP-NADPH incubations. Enzymatic lipid peroxidation in the microsomes was studied by using Fe^{3+} complex and NADPH. This system makes use of cytochrome P450 as the ultimate reductant (Minotti 1992). Peroxidation was induced in the incubation mixture containing microsomes (0·4 mg protein) and various concentrations of the test compounds, 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 of the 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.

Spectroscopic studies

Solution containing curcuminoid (25 μM) and various concentrations of Fe^{2+} or Fe^{3+} (2–50 μM) was scanned between 400 and 500 nm using a Shimadzu UV-240 Graphicord spectrophotometer.

Table 1. Effect of curcuminoids on Fe^{2+}, Fe^{3+} and Fe^{3+}-ADP-ascorbic acid-stimulated lipid peroxidation in rat brain homogenates and Fe^{2+}, Fe^{3+} and Fe^{3+}-ADP-NADPH-induced lipid peroxidation in liver microsomes.

Concn (μM)	Inhibition of thiobarbituric acid reactive substances (%)					
	Brain homogenates			Liver microsomes		
	Fe^{2+}	Fe^{3+}	Fe^{2+}-ADP-ascorbate	Fe^{2+}	Fe^{3+}	Fe^{2+}-ADP-NADPH
Curcumin						
0·25	3·2 ± 1·0	9·4 ± 0·9	10·1 ± 0·9	1·0 ± 0·3	0·5 ± 0·1	0·5 ± 0·1
0·5	26·4 ± 1·4	39·1 ± 1·1	40·3 ± 1·0	5·1 ± 0·9	4·3 ± 1·2	4·0 ± 1·0
1	39·3 ± 1·3	60·9 ± 1·8	63·7 ± 1·5	13·9 ± 0·9	13·2 ± 1·3	10·4 ± 1·1
5	76·1 ± 1·9	83·2 ± 1·7	85·4 ± 1·7	48·7 ± 1·7	44·3 ± 1·6	39·7 ± 1·4
10	93·5 ± 2·0	96·4 ± 1·6	96·9 ± 2·1	73·2 ± 1·4	69·1 ± 1·4	60·4 ± 1·9
20	100	100	100	99·0 ± 1·7	99·5 ± 1·9	98·7 ± 2·0
Demethyoxycurcumin						
0·25	2·4 ± 1·1	10·1 ± 0·9	10·8 ± 1·0	0·5 ± 0·1	0·2 ± 0·0	0·4 ± 0·0
0·5	27·1 ± 1·3	37·4 ± 1·1	42·4 ± 1·8	4·8 ± 0·8	3·5 ± 0·7	3·0 ± 0·8
1	37·9 ± 1·4	60·1 ± 1·4	65·8 ± 1·7	14·2 ± 1·1	12·2 ± 1·4	11·2 ± 1·2
5	78·0 ± 2·1	84·1 ± 2·2	84·3 ± 2·1	45·1 ± 1·7	45·1 ± 1·7	37·4 ± 1·3
10	92·6 ± 2·3	93·9 ± 2·0	95·4 ± 2·3	70·4 ± 2·5	69·4 ± 2·0	58·3 ± 2·3
20	100	100	100	98·2 ± 1·8	97·4 ± 1·5	95·7 ± 2·4
Bisdemethoxycurcumin						
0·25	2·0 ± 0·8	8·2 ± 1·0	8·9 ± 0·8	0·2 ± 0·0	0·4 ± 0·2	0·6 ± 0·2
0·5	27·3 ± 1·4	37·2 ± 1·3	37·3 ± 1·8	4·0 ± 0·5	3·2 ± 4·1	3·5 ± 1·0
1	37·2 ± 1·7	57·3 ± 1·7	60·2 ± 1·7	12·4 ± 0·5	11·4 ± 1·7	9·1 ± 1·1
5	75·2 ± 2·3	82·4 ± 2·1	86·1 ± 2·3	43·2 ± 1·8	45·1 ± 1·8	40·1 ± 1·7
10	91·3 ± 2·1	97·1 ± 2·4	95·4 ± 2·1	75·2 ± 1·9	66·4 ± 2·0	58·3 ± 2·0
20	100	100	100	97·2 ± 2·1	97·5 ± 1·9	98·7 ± 2·2
Acetylcurcumin						
0·25	2·1 ± 0·4	10·2 ± 0·7	9·4 ± 0·7	0·4 ± 0·0	0·2 ± 0·0	0·5 ± 0·2
0·5	24·2 ± 1·7	36·5 ± 1·1	38·0 ± 1·7	3·3 ± 0·7	3·0 ± 1·0	4·0 ± 0·5
1	39·0 ± 1·8	63·2 ± 1·5	65·3 ± 1·8	14·2 ± 1·0	12·7 ± 1·2	10·4 ± 1·2
5	78·1 ± 1·8	85·5 ± 2·3	84·4 ± 2·4	40·0 ± 1·5	46·4 ± 1·3	42·4 ± 1·0
10	94·1 ± 1·9	98·9 ± 2·0	97·9 ± 2·3	73·4 ± 1·5	65·4 ± 1·8	63·1 ± 2·3
20	100	100	100	98·4 ± 1·7	96·1 ± 2·1	97·4 ± 2·5

Percent inhibition was calculated by comparison with control experiments without curcuminoid and expressed as mean ± s.e. (n = 3). Values are the average of one representative experiment in triplicate.

Results and Discussion

The results show that the three natural curcuminoids are potent inhibitors of iron-stimulated lipid peroxidation in rat brain homogenates and rat liver microsomes (Table 1). These compounds were more active than α-tocopherol in all the models tested, and their activity profile shows that the three compounds are almost equally active (Tables 2, 3).

Curcumin has been studied extensively for its antioxidant properties. The phenolic and the methoxy group on the phenyl ring and the 1,3-diketone system are the two important structural features that contribute to its antioxidant properties. It has been suggested that the antioxidant activity of the phenolic group increases with a methoxy at the ortho position (Cuvelier et al 1992). Toda et al (1988) studied the action of curcuminoids on the haemolysis and lipid peroxidation of mouse erythrocytes induced by hydrogen peroxide. Those authors found that

Table 2. Inhibitory activities of curcuminoids and α-tocopherol against Fe^{2+}, Fe^{3+} and Fe^{3+}-ADP-ascorbate-induced lipid peroxidation in rat brain homogenate.

System	IC50 (μM)				
	Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin	Acetylcurcumin	α-Tocopherol
Fe^{2+}	1·6	1·7	1·6	1·6	8·3
Fe^{3+}	1·3	1·1	0·9	1·0	7·4
Fe^{3+}-ADP-ascorbate	0·9	1·0	1·0	1·0	10·3

Data are mean of triplicate determinations.

Table 3. Inhibitory activities of curcuminoids and α-tocopherol against Fe^{2+}, Fe^{3+} and Fe^{3+}-ADP-NADPH-induced lipid peroxidation in rat liver microsomes.

System	IC50 (μM)				
	Curcumin	Demethoxycurcumin	Bisdemethoxycurcumin	Acetylcurcumin	α-Tocopherol
Fe^{2+}	4·3	4·9	4·6	5·0	7·2
Fe^{3+}	4·2	5·3	5·5	5·5	18·2
Fe^{3+}-ADP-NADPH	7·5	8·8	8·1	6·5	21·9

Data are mean of triplicate determinations.

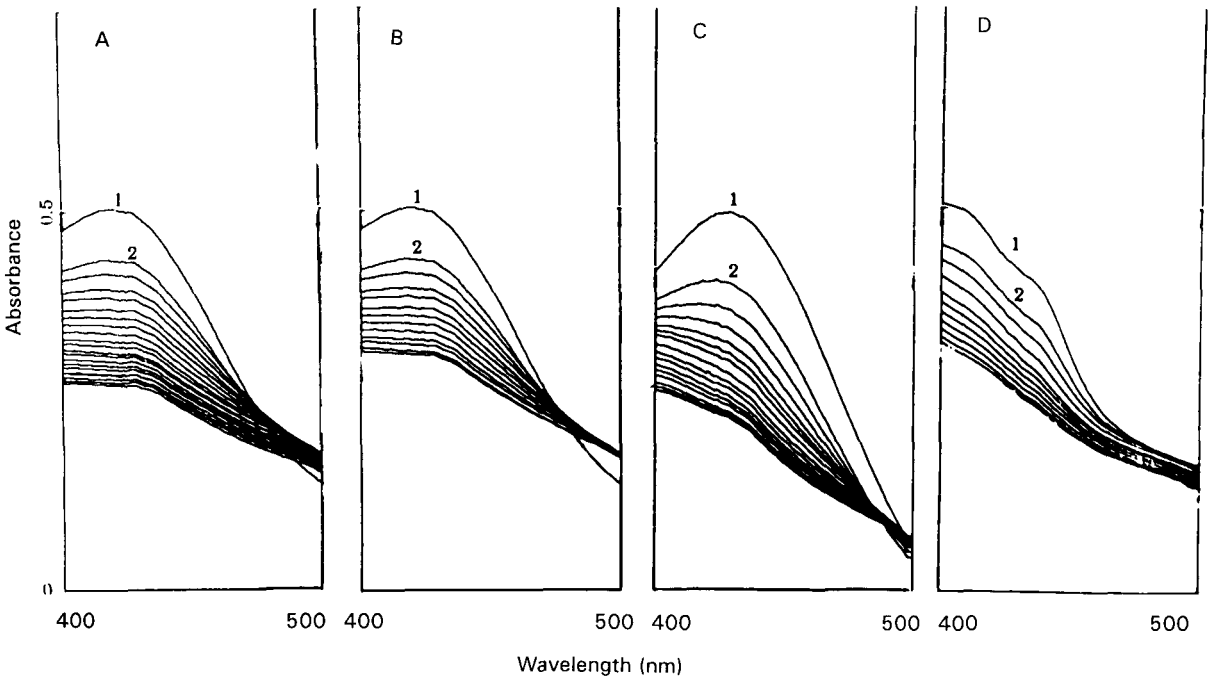

FIG. 2. Spectra of curcuminoids in the presence of Fe^{2+}. A. curcumin; B. demethoxycurcumin; C. bisdemethoxycurcumin; D. acetylcurcumin. In the above spectra, curve 1 refers to the spectra of curcuminoid (25 μM), curve 2 refers to the spectra in the presence of 3 μM Fe^{2+}. The remaining curves were obtained by the addition of higher concentrations of Fe^{2+}.

curcumin was more active than the demethylated analogues. However, in the present study, we found that the three curcuminoids are almost equally active. Thus, in the case of iron-catalysed lipid peroxidation inhibition by curcumin, the methoxy group may not play a major role.

To assess the importance of the phenolic group, we studied the acetyl derivative where the phenolic group is blocked by the acetyl group; acetylcurcumin and curcumin were equally active indicating that the phenolic group may not be essential for the antioxidant properties of curcumin observed in the present study. Other authors have come to similar conclusions by studying synthetic non-phenolic analogues of curcumin (Conney et al 1990; Tonnesen & Greenhill 1992). Tonnesen & Greenhill (1992) found that 5-hydroxy-1,7 diphenyl-1,4,6-heptatriene-3-one, a curcumin analogue lacking the phenolic and methoxy groups, is as active as curcumin in scavenging oxygen free radicals and in other redox properties. Conney et al (1990) used similar analogues for the inhibitory effect on 12-*o*-tetradecanoyl phorbol-13-acetate-induced tumour promotion in mouse skin. They found that the free phenolic group on the benzene ring in curcumin is not required for the antitumour activity. Further they found that chlorgenic acid, caffeic acid and ferulic acid which do not contain a 1,3-diketone system were less active.

Almost similar activity shown by all the curcuminoids and acetylcurcumin in the present study suggests that the phenolic group or the methoxy group on the benzene ring is not important for the inhibition of iron-catalysed lipid peroxidation. The 1,3-diketone system is a potent ligand for metals such as iron. From the spectral studies it is clear that the curcuminoids are capable of interaction with Fe^{2+} (Fig. 2). Similar results were obtained with Fe^{3+} (data not shown).

References

Ammon, H. P. T., Wahl, M. A. (1991) Pharmacology of curcumin. Planta Medica 57: 1–7

Braughler, J. M., Duncan, L. A., Chase, R. L. (1986) The involvement of iron in lipid peroxidation: importance of ferrous to ferric ratios in initiation. J. Biol. Chem. 261: 10282–10292

Conney, A. H., Lysz, T., Ferraro, T., Abidi, T. F., Manchand, P. S., Laskin, J. D., Haung, M. T. (1990) Inhibitory effect of curcumin and some related dietary compounds on tumour promotion and arachidonic acid metabolism in mouse skin. In: Weber, G. (ed.) Advances in Enzyme Regulation. Vol. 31, Pergamon Press, New York, pp 385–396

Cuvelier, M. E., Richard, H., Berset, C. (1992) Comparison of antioxidative activity of some acid-phenols: structure-activity relationship. Biosci. Biotech. Biochem. 56: 324–325

Ernster, L., Nordenbrand, K. (1967) Microsomal lipid peroxidation. In: Estabrook, R. W., Pullman, M. E. (eds) Methods in Enzymology. Vol. X, Academic Press, New York, pp 574–576

Halliwell, B., Gutteridge, J. M. C. (1990) Role of free radicals and catalytic metals in human disease: an overview. In: Packer, L., Glazer, A. N. (eds) Methods in Enzymology. Vol. 186, Academic Press, New York, pp 1–85

Kunchandy, E., Rao, M. N. A. (1989) Effect of curcumin on hydroxyl radical generation through Fenton reaction. Int. J. Pharm. 57: 173–176

Kunchandy, E., Rao, M. N. A. (1990) Oxygen radical scavenging activity of curcumin. Int. J. Pharm. 58: 237–240

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Minotti, G. (1992) The role of endogenous nonheme iron in microsomal redox reactions. Arch. Biochem. Biophys. 297: 189–198

Pabon, H. J. J. (1964) A synthesis of curcumin and related compounds. Rec. Trav. Chim. Pays-Bas. 83: 279–386

Roughley, P. J., Whiting, A. D. (1973) Experiments in the biosynthesis of curcumin. J. Chem. Soc. 2379–2388

Sharma, O. P. (1976) Antioxidant activity of curcumin and related substances. Biochem. Pharmacol. 25: 1811–1812

Sharma, S. L., Muhktar, H., Sharma, S. K., Murti, C. R. K. (1972) Lipid peroxide formation in experimental inflammation. Biochem. Pharmacol. 21: 1210–1214

Sreejayan, Rao, M. N. A. (1993) Curcumin inhibits iron dependent lipid peroxidation. Int. J. Pharm. 100: 93–97

Srimal, R. C. (1987) Curcumin. Drugs Future 12: 331–333

Sugioka, K., Shimosegawa, Y., Nakano, M. (1987) Estrogen as natural antioxidants of membrane phospholipid peroxidation. FEBS Lett. 210: 37–39

Susan, M., Rao, M. N. A. (1991) Interaction of curcumin with glutathione. Int. J. Pharm. 76: 257–259

Susan, M., Rao, M. N. A. (1992) Induction of glutathione-S-transferase activity by curcumin in mice. Arzneim. Forsch. 42: 962–964

Toda, S., Ohnishi, M., Kimura, M., Nakashima, K. (1988) J. Ethnopharmacol. 23: 105–108

Tonnesen, H. H., Greenhill, J. V. (1992) Studies on curcumin and curcuminoids XXII: curcumin as a reducing agent and radical scavenger. Int. J. Pharm. 87: 79–87

Tonnesen, H. H., Smistad, G., Agren, T., Karlsen, J. (1993) Studies on curcumin and curcuminoids. XXII: Effects of curcumin on liposomal lipid peroxidation. Int. J. Pharm. 90: 221–228

Unnikrishnan, M. K., Rao, M. N. A. (1992) Curcumin inhibits nitrite induced methemoglobin formation. FEBS Lett. 301: 195–196

Wiseman, H., Laughton, M. J., Arnstein, H. R. V., Cannon, M., Halliwell, B. (1990) The antioxidant action of tamoxifen and its metabolites: inhibition of lipid peroxidation. FEBS Lett. 263: 192–194