International Journal of Pharmaceutics, 58 (1990) 237–240
Elsevier

IJP 02033

Note

Oxygen radical scavenging activity of curcumin

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(Received 21 October 1989)
(Accepted 8 November 1989)

Key words: Curcumin; Free-radical scavenger; Hydroxyl radical; Superoxide scavenger

Summary

Curcumin, a potent anti-inflammatory agent, has been studied for its ability to scavenge reactive oxygen radicals which are implicated in inflammation. Studies showed that it is a good scavenger of hydroxyl radical at high concentrations but at low concentrations activated the Fenton system to generate an increased amount of hydroxyl radical. Curcumin was also studied for superoxide scavenging activity and was found to be a potent scavenger.

In our earlier communication we reported the ability of curcumin to generate hydroxyl radical through the Fenton reaction by reducing Fe^{3+} to Fe^{2+} (Elizabeth and Rao, 1989). Curcumin is a potent anti-inflammatory agent (Srimal, 1987). Many anti-inflammatory agents are known to act through scavenging of oxygen radicals (Roberfroid et al., 1987; Santrucek and Krepelka, 1988). In our studies on styryl ketones, we found a good correlation between oxygen radical scavenging and anti-inflammatory activities (Lovina et al., 1989). Since curcumin is also a styryl ketone derivative, we were interested in its oxygen radical scavenging activity. Here, we have studied the hydroxyl radical and superoxide scavenging activities of curcumin at various concentrations.

Curcumin, *p*-nitrosodimethylaniline (pNDA), thiobarbituric acid, nitroblue tetrazolium (NBT) and 2-deoxy-D-ribose were from Sigma, Co. All other chemicals were of analytical grade.

Curcumin (5 mg) was dissolved in 5 ml cold NaOH (0.1%) and immediately diluted to the required concentration with phosphate buffer, pH 7.4 (20 mM).

Hydroxyl radical scavenging as measured by the inhibition of *p*-nitrosodimethylaniline (pNDA) bleaching: hydroxyl radical generated through Fenton reaction can bleach pNDA specifically. Scavenging activity was measured by the extent of inhibition of bleaching in the presence of curcumin. To a reaction mixture containing ferric chloride (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), H_2O (2 mM) and pNDA (0.01 mM) in phosphate buffer, pH 7.4 (20 mM), were added various concentrations of curcumin to give a final volume of 3 ml. Absorbance was measured at 440 nm. Percentage scavenging was calculated from the control where no curcumin was present. Experiments were performed in triplicate.

Hydroxyl radical scavenging as measured by the inhibition of deoxyribose degradation: the de-

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gradation of deoxyribose by hydroxyl radical generated as above was measured colorimetrically in the presence and absence of curcumin (Lovina et al., 1989). To the reaction mixture containing deoxyribose (3 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), H_2O_2 ((2 mM) in phosphate buffer, pH 7.4 (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%) were added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance measured at 532 nm.

Scavenging of superoxide radical: Superoxide was generated according to the alkaline DMSO method (Hyland et al., 1983). The reduction of NBT by superoxide was determined in the presence and absence of curcumin. To the reaction mixture containing NBT (0.1 mg) and curcumin at various concentrations was added alkaline DMSO (1 ml, 1% water, 5 mM NaOH) to give a final volume of 1.4 ml and the absorbance was evaluated at 560 nm.

Fig. 1 shows plots of the rate of generation of hydroxyl radical in the presence and absence of curcumin as measured by the bleaching of pNDA. In both cases, the plots are linear with time. The initial rate constant for the generation of hydroxyl radical in the absence of curcumin was found to

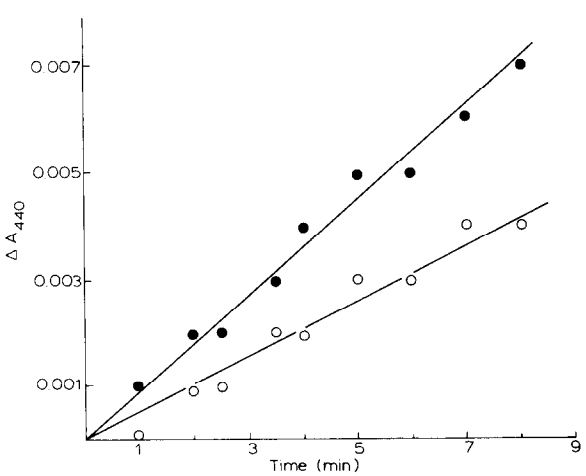

Fig. 1. Rate of scavenging of hydroxyl radical by curcumin. Control (●), 15 μM curcumin (○).

TABLE 1

Hydroxyl radical scavenging of curcumin as measured by the bleaching of p-nitrosodimethylaniline

Hydroxyl radical was generated through the Fenton reaction. Scavenging was measured by the inhibition of bleaching of *p*-nitrosodimethylaniline.

[Curcumin] (μM)	Scavenging (%) (±S.E.)
27.00	62.5 ±5.6
5.40	52.7 ±2.9
2.70	39.9 ±2.2
1.35	33.3 ±3.3
0.61	−7.15 ±2.3 (activation) *

* Activation: increased bleaching was observed.

be $K = 0.005156$. In the presence of curcumin the rate was reduced to $K = 0.003059$.

The effect of curcumin at various concentrations on the bleaching of pNDA was studied (Table 1). At high concentrations curcumin protected pNDA from bleaching in a dose-dependent manner. This protection can be attributed to the scavenging action of curcumin on the hydroxyl radical. However, at low concentration (0.61 μM) there was increased bleaching of pNDA compared to the control. This suggested activation of the Fenton reaction resulting in increased generation of hydroxyl radical. To confirm this observation, another method was selected where generation of hydroxyl radical can be measured by the degradation of deoxyribose (Halliwell et al., 1987). Table 2 lists data on the effect of curcumin on the degradation of deoxyribose by hydroxyl radical. In this method also curcumin protected deoxyribose from degradation, suggesting scavenging of hydroxyl radical. At low concentration (1.35 μM), in contrast, there occurred increased degradation indicative of increased generation of hydroxyl radical.

From the above observations it is clear that curcumin has a dual action. At higher concentrations it scavenges hydroxyl radical, whereas at lower levels, it activates the Fenton reaction, resulting in increased hydroxyl radical generation. We have previously reported that curcumin activates the Fenton reaction by reducing Fe^{3+} to Fe^{2+} (Elizabeth and Rao, 1989). In the present

TABLE 2

Hydroxyl radical scavenging of curcumin as measured by the deoxyribose method

Hydroxyl radical was generated through the Fenton reaction. Scavenging was measured by the inhibition of deoxyribose degradation in the presence of curcumin.

[Curcumin] (μM)	Scavenging (%) ($\pm$S.E.)
54.00	33.3 ± 1.3
27.00	6.1 ± 0.9
5.40	4.6 ± 0.6
2.70	1.3 ± 0.1
1.35	-10.6 ± 1.1 (activation) *

* Activation: increased degradation of deoxyribose was observed.

study, the Fenton reaction is carried out in the presence of ascorbic acid which acts as a reducing agent. Curcumin may exert its effect in a synergistic manner at low concentrations, whereas at higher values the of acting as a property reducing agent becomes saturated and its scavenging activity predominates. Ascorbic acid is also known to behave similarly. It can activate the Fenton reaction, but at different concentrations it can also scavenge oxygen radicals (Santrucek and Krepelka, 1988).

Although hydroxyl radical is more reactive and toxic to tissues than is superoxide radical, it has been suggested that the role played by superoxide is more important for inflammation (Petrone et al., 1980; Oyanagui, 1982. This is mainly due to the fact that hydroxyl radical scavengers, such as mannitol, benzoate, etc., are devoid of anti-inflammatory activity (Oyanagui, 1982), whereas superoxide dismutase which scavenges superoxide shows a considerable extent (Petrone et al., 1980). Hence, the effect of curcumin as superoxide scavenger was studied (Table 3). Perusal of Table 3 shows that curcumin is a potent scavenger of superoxide radical. Scavenging is dose dependent. The potent anti-inflammatory activity of curcumin may be due to its superoxide scavenging. Also, in our studies on styryl ketones, we observed a better correlation between anti-inflammatory activity and superoxide scavenging than with hydroxyl radical scavenging (Lovina et al., 1989).

TABLE 3

Scavenging of superoxide radical by curcumin

Superoxide was generated by the alkaline DMSO method. Scavenging was measured by the inhibition of NBT reduction in the presence of curcumin.

[Curcumin] (μM)	Scavenging (%) $\pm$(S.E.)
1.35	6.3 ± 0.4
2.70	20.9 ± 0.5
27.00	30.0 ± 0.6
54.00	39.0 ± 0.9
270.00	76.8 ± 1.4

In conclusion, the present study shows that curcumin act as hydroxyl radical scavenger at higher concentrations and at lower concentration it activates the Fenton reaction resulting in increased generation of hydroxyl radical. Curcumin was also found to be a potent scavenger of superoxide radical and this property may be responsible for the good anti-inflammatory activity of curcumin.

Acknowledgement: The authors thank Prof. P. Gundu Rao, Principal, College of Pharmaceutical Sciences, K.M.C., Manipal, for providing all the facilities.

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