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Nitric Oxide Scavenging by Curcuminoids

SREEJAYAN AND M. N. A. RAO

Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Manipal 576 119, India

Abstract

Because curcumin, a compound with anti-inflammatory and anticancer activity, inhibits induction of nitric oxide synthase in activated macrophages and has been shown to be a potent scavenger of free radicals we have investigated whether it can scavenge nitric oxide directly.

Curcumin reduced the amount of nitrite formed by the reaction between oxygen and nitric oxide generated from sodium nitroprusside. Other related compounds, e.g. demethoxycurcumin, bisdemethoxycurcumin and diacetylcurcumin were as active as curcumin, indicating that the methoxy and the phenolic groups are not essential for the scavenging activity.

The results indicate curcumin to be a scavenger of nitric oxide. Because this compound is implicated in inflammation and cancer, the therapeutic properties of curcumin against these conditions might be at least partly explained by its free-radical scavenging properties, including those toward nitric oxide.

Curcumin (diferuloyl methane, CAS 458-37-7) is the major colouring pigment present in the rhizomes of *Curcuma longa* a spice widely used in Indian cooking (Ammon & Wahl 1991). It possesses many therapeutic properties including anti-inflammatory and anticancer activities (Srimal 1987). Our earlier studies have shown that curcumin is a powerful scavenger of the superoxide anion (Kunchandy & Rao 1990), the hydroxyl radical (Kunchandy & Rao 1989), nitrogen dioxide (Unnikrishnan & Rao 1995) and the nitrogen-centred stable free radical 1,1-diphenyl-2-picrylhydrazyl (Sreejayan & Rao 1996). It also protects DNA against singlet-oxygen-induced strand break (Subramanian et al 1994), lipids from peroxidation (Sreejayan & Rao 1993) and oxyhaemoglobin from nitrite-induced oxidation (Unnikrishnan & Rao 1992). Recently, Brouet & Ohshima (1995) reported that curcumin inhibits induction of nitric oxide synthase in activated macrophages. Joe & Lokesh (1994) also reported similar results using rat peritoneal macrophages. In both the reports it was shown that the amount of nitric oxide generated was significantly reduced in the presence of curcumin. Because nitric oxide is a free radical and curcumin is a potent scavenger of free radicals including nitrogen centred free radicals such as 1-diphenyl-2-picrylhydrazyl and nitrogen dioxide (Unnikrishnan & Rao 1995; Sreejayan & Rao 1993), we found it interesting to study whether curcumin has the ability to scavenge nitric oxide directly.

Materials and Methods

Materials

Curcumin and bisdemethoxycurcumin were synthesized as described by Pabon (1964). Demethoxycurcumin was isolated from the rhizomes of *Curcuma longa* by preparative thin layer chromatography. Diacetylcurcumin was prepared from curcumin as described elsewhere (Roughley & Whiting 1973). The identities of all the compounds were confirmed by melting point, elemental analysis (C and H), and spectral studies (IR, NMR and mass spectra). Sodium nitroprusside, sulphanilamide, naphthylethylenediamine dihydrochloride were of analytical grade. The structures of curcumin and related compounds are shown in Fig. 1.

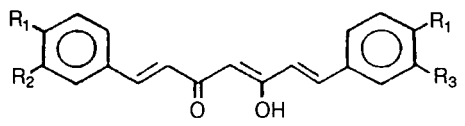
Correspondence: M. N. A. Rao, Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Manipal 576 119, India.

Nitric oxide production from sodium nitroprusside: assay of nitrite production

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction as described previously (Green et al 1982; Marcocci et al 1994a). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (Green et al 1982; Marcocci et al 1994a) which interacts with oxygen to produce nitrite ions which can be estimated by use of the Greiss reagent (Marcocci et al 1994b). Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Marcocci et al 1994b). Sodium nitroprusside (5 mM) in phosphate-buffered saline was mixed with different concentrations of curcumin dissolved in alcohol and incubated at 25°C for 150 min. A control experiment without test compound but with the equivalent amount of alcohol was conducted in an identical manner. At intervals, samples (0·5 mL) of the incubation solution were removed and diluted with 0·5 mL of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0·1% naphthylethylenediamine dihydrochloride). The absorbance of the chromaphore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite treated in the same way with Griess reagent.

Results

Incubation of solutions of sodium nitroprusside in phosphate-buffered saline at 25°C for 2 h resulted in linear time-depen-



	R_1	R_2	R_3
Curcumin	OH	OMe	OMe
Demethoxycurcumin	OH	OMe	H
Bisdemethoxycurcumin	OH	H	H
Diacetylcurcumin	OAc	OME	OMe

FIG. 1. The structures of curcumin and related compounds.

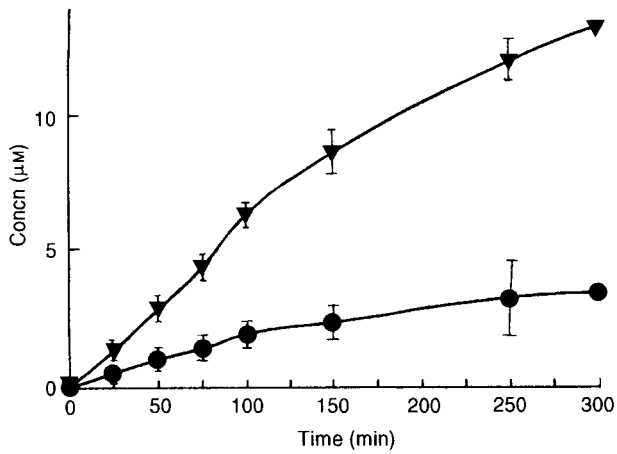


FIG. 2. Production of nitrite from solution of 5 mM sodium nitroprusside in the absence (▼) or presence (●) of 25 μM curcumin. Sodium nitroprusside (5 mM) in PBS was mixed with curcumin (25 μM) dissolved in alcohol and incubated at 25°C for 150 min. At intervals, samples (0·5 mL) of incubation solution were removed and diluted with 0·5 mL of Griess reagent and the absorbance was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite treated in the same way with Griess reagent.

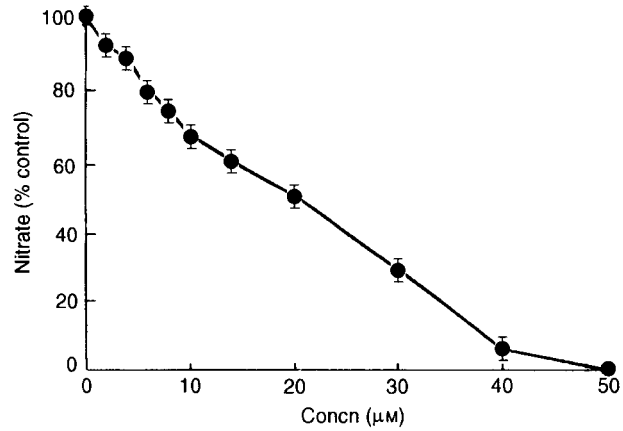


FIG. 3. The effect of curcumin on the accumulation of nitrite upon decomposition of sodium nitroprusside (5 mM). Incubation time 150 min; temperature 25°C.

Table 1. Effect of curcuminoids on the production of nitrite from sodium nitroprusside.

Compound	IC50 (μM)
Curcumin	20·4 ± 2·4
Demethoxycurcumin	18·2 ± 2·1
Bisdemethoxycurcumin	19·1 ± 2·7
Diacetylcurcumin	17·9 ± 1·9

Data are means ± s.e.m. (n = 3).

dent nitrite production which was reduced by the presence of 25 μM curcumin (Fig. 2). The scavenging of nitric oxide by curcumin was concentration-dependent (Fig. 3); at concentrations of 20·4 and 50 μM curcumin scavenged 50% (approximately) and 100%, respectively, of the nitric oxide generated by 150 min incubation. Addition of 50 μM curcumin to 10–50 μM standard solutions of potassium nitrite did not change their absorbance upon treatment with Griess reagent, indicating that curcumin does not interfere with the nitrite detection assay nor does it directly interact with nitrite.

We also studied demethoxycurcumin and bisdemethoxycurcumin, which also occur naturally, and diacetylcurcumin for their nitric oxide-scavenging properties (Table 1). These compounds were as active as curcumin, indicating that neither the methoxy group nor the phenolic group was essential for nitric oxide scavenging activity.

Discussion

This study demonstrates that curcumin is a potent scavenger of nitric oxide. Nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. Curcumin inhibits nitrite formation by competing with oxygen to react with nitric oxide. It has previously been reported that curcumin inhibits the generation of nitric oxide from activated macrophages as measured by the nitrite method (Joe & Lokesh 1994). This inhibition might also be a result of direct scavenging of nitric oxide by curcumin. Brouet & Ohshima (1995), however, also showed that curcumin inhibits the induction of nitric oxide synthase by activated macrophages as measured by the amount of citrulline formed. It thus appears that curcumin has the ability both to scavenge nitric oxide directly and also to inhibit its biosynthesis. Our studies further demonstrate that the methoxy and the phenolic groups are not essential for nitric oxide-scavenging activity, because demethoxycurcumin, bisdemethoxycurcumin and diacetylcurcumin were almost as active as curcumin. This observation is interesting because the antioxidant properties of curcumin are generally attributed to its phenolic nature (Toda et al 1988). We have previously observed that for superoxide and 1,1-diphenyl-2-picrylhydrazyl scavenging, the order of activity was : curcumin > demethoxycurcumin > bisdemethoxycurcumin > diacetylcurcumin (almost inactive) (Sreejayan & Rao 1996). For inhibition of iron-catalyzed lipid peroxidation, however, all four compounds were equally active (Sreejayan & Rao 1994). Thus the 1,3-diketone system might be playing an important role in nitric oxide scavenging by curcumin.

The nitric oxide scavenging ability of curcumin further expands the role of curcumin as a potent antioxidant (Sree-

jayan & Rao 1994). In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions (Marletta 1989; Moncada et al 1991). The therapeutic properties of curcumin in these and other pathological conditions might be explained, at least partly, by its ability to scavenge nitric oxide.

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