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Curcumin inhibits nitrite-induced methemoglobin formation

M.K. Unnikrishnan and M.N.A. Rao

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

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Curcumin protects hemoglobin from nitrite-induced oxidation to methemoglobin. The protection is not observed when curcumin is added after the autocatalytic stage of the oxidation of hemoglobin by nitrite. The ability of curcumin to scavenge superoxide may be responsible since superoxide is implicated in promoting the autocatalytic stage of oxidation of hemoglobin by nitrite.

Curcumin; Methemoglobin formation inhibition; Nitrite-induced methemoglobin; Antioxidant

1. INTRODUCTION

Curcumin is a potent scavenger of reactive oxygen species like superoxide anions and hydroxyl radicals [1]. It is also capable of reducing ferric ions to ferrous state [2]. Its antioxidant property is further shown by its capacity to inhibit lipid peroxidation in rat brain homogenate [3].

The oxidation of hemoglobin to methemoglobin by nitrite has been widely studied [4–6]. The formation of methemoglobin occurs in two stages. The first is a slow stage, and the second is a rapid autocatalytic stage [4–5]. Superoxide has been implicated in the autocatalytic stage of the oxidation [6] which is inhibited by superoxide dismutase [4]. Since curcumin is a potent scavenger of superoxide, the present work was undertaken to ascertain the ability of curcumin to inhibit nitrite-induced oxidation of hemoglobin to methemoglobin.

2. MATERIALS AND METHODS

2.1. *Materials*

Curcumin was obtained from Sigma Chemical Co. and sodium nitrite (from Glaxo India) was of analytical grade. Human blood, collected into acid-citrate-dextrose, was from the blood bank of Kasturba Hospital, Manipal.

2.2. *Nitrite-induced methemoglobin in hemolysate*

Blood samples were centrifuged (2,500 × *g*, 20 min) to remove plasma and the buffy coat of white cells. The erythrocytes obtained were washed thrice with phosphate-buffered saline. The washed cells were lysed by suspending in 20 vols. of 20 mM phosphate buffer, pH 7.4. The hemolysate was centrifuged at 25,000 × *g* for 60 min to remove the membrane, and then diluted to give a 150 μM concentration of oxyhemoglobin. The reaction was initiated by the addition of sodium nitrite (final conc. 0.6 mM) to the solution of hemolysate and the formation of methemoglobin was measured by monitoring absorbance at 631 nm using a Shimadzu Graphicord UV 240 Spectrophotometer. Curcumin was added either before or at various time-intervals after the addition of nitrite. Control experiments were conducted without curcumin and all experiments were in triplicate and were repeated many times. The data given are for one such set.

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

2.3. *Nitrite-induced methemoglobin in erythrocytes*

The washed erythrocyte suspension was incubated with curcumin for 30 min followed by addition of sodium nitrite (final conc. 0.6 mM) for further 120 min. The suspension was centrifuged at 2,500 × *g* for 20 min to remove the excess curcumin and nitrite. The cells were washed thrice with phosphate-buffered saline and lysed with 20 mM phosphate buffer, pH 7.4. The lysate was centrifuged at 25,000 × *g* for 60 min, and the clear supernatant was removed and absorbance at 631 nm was measured. Control experiments were conducted without the addition of curcumin. The extent of methemoglobin formation by nitrite was calculated after taking into consideration the amount of methemoglobin already present in the erythrocyte before the addition of nitrite. All experiments were in triplicate and repeated several times. The data given represent one such set.

3. RESULTS

3.1. *Methemoglobin formation in hemolysate*

Nitrite causes a rapid oxidation of hemoglobin to methemoglobin. In the presence of curcumin, the oxidation process was delayed in a dose-dependent manner (Table I). The time required to convert 50% of the available hemoglobin to methemoglobin was 6.7 min in the absence of curcumin, whereas with 20 μM curcumin present the time was increased to 45.2 min.

Fig. 1 describes the effect of curcumin on the time-course of nitrite oxidation of hemoglobin. Without curcumin, the time-course of oxidation shows a characteristic pattern of slow initial transformation followed by a rapid autocatalytic process. When curcumin was added along with nitrite, i.e. at 0 min, the formation of methemoglobin was inhibited to a great extent. Addition of curcumin 5 min after nitrite also resulted in protection of the hemoglobin from oxidation to an appreciable extent. However, when curcumin was added

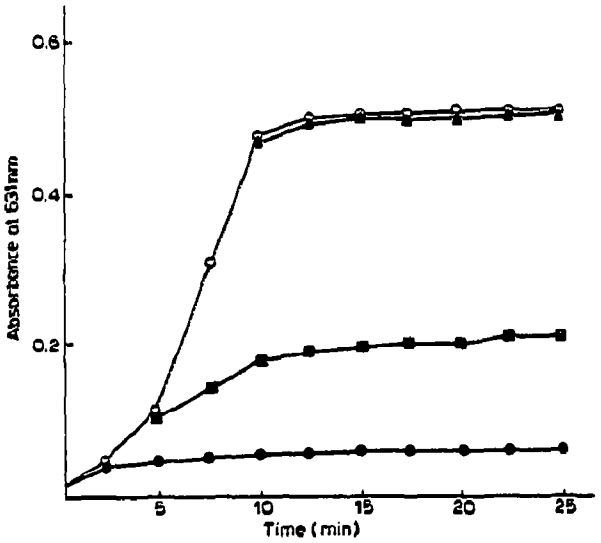

Fig. 1. Time course of methemoglobin formation. Hemolysate containing hemoglobin (150 μM) was treated with nitrite (0.6 mM). Curcumin (20 μM) was added at 0 min (●), at 5 min (■), and at 10 min (▲). Control without curcumin (○).

at the end of the autocatalytic stage, i.e. at 10 min, no protection was observed.

3.2. *Methemoglobin formation in intact erythrocytes*

Curcumin was able to inhibit the formation of methemoglobin in intact erythrocytes (Table II). There was a dose-dependent inhibition when erythrocytes were incubated with curcumin before addition of nitrite. However, there was no protection when curcumin was added after nitrite. Thus, the erythrocytes behaved in a similar manner to that of the hemolysate.

4. DISCUSSION

The present study has shown that curcumin can protect hemoglobin from oxidation by sodium nitrite both in hemolysate and in intact erythrocytes. However, it

Table I

Inhibition of nitrite-induced methemoglobin formation by curcumin in hemolysate

Curcumin (μM)	Time to form 50% methemoglobin* min (± SE)
Control	6.7 (± 2.0)
0.2	9.8 (± 1.8)
2.0	13.2 (± 1.2)
4.0	18.3 (± 2.0)
20.0	45.2 (± 6.0)

*Hemolysate containing 150 μM hemoglobin was incubated with curcumin for 10 min before the addition of nitrite (0.6 mM) and absorbance measured at 631 nm at 1 min intervals. Results are means ± standard error (SE); n = 3

Table II

Inhibition of nitrite-induced methemoglobin formation in erythrocytes by curcumin

Curcumin (μM)	Inhibition* % (± SE)
8	8.6 (± 2.6)
20	16.0 (± 6.2)
80	25.0 (± 8.2)
400	51.2 (± 10.2)

*Erythrocytes were incubated with curcumin for 30 min followed by nitrite (1.8 μM) for a further 120 min. After cell lysis, the levels of methemoglobin were measured by absorbance at 631 nm. Percent inhibition was calculated compared to control. Results are mean values ± standard error (SE); n = 3.

did not to reverse the effect of nitrite if added at a later stage. It is well established that oxidation of hemoglobin takes place in two stages. There is a slow initial stage followed by a rapid autocatalytic stage, which carries the reaction to completion [4]. Curcumin is able to prevent the onset of the autocatalytic stage. Since superoxide is implicated in the autocatalytic stage [4] and the fact that curcumin is a potent scavenger of superoxide [1], suggests that the protective action of curcumin is by scavenging superoxide generated during the oxidation. Superoxide dismutase also inhibits the onset of the autocatalytic stage [4]. Direct interaction between nitrite and curcumin as a reason for protection is ruled out because the concentration of curcumin causing protection is very low (<20 μM) compared to the nitrite concentration (0.6 mM). Although curcumin can reduce ferric ions to the ferrous state, it fails to reverse the oxidation of hemoglobin, suggesting that protection is not due to reduction of methemoglobin to hemoglobin. Many antioxidants like ascorbic acid, uric acid, 3-ribosyl uric acid, and glutathione protect hemoglobin from oxidation by nitrite [7]. These antioxidants also inhibit the onset of the autocatalytic stage of nitrite if added at a later stage [7]. Thus, the effect of curcumin may be similar to these antioxidants in protecting hemoglobin from nitrite ions.

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