Original Article

Prevention of H_2O_2-induced red blood cell lipid peroxidation and hemolysis by aqueous extracted turmeric

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The protective effect of the aqueous extracted turmeric antioxidant protein on H_2O_2-induced red blood cell lipid peroxidation and hemolysis was investigated. Turmeric antioxidant protein inhibited lipid peroxidation and hemolysis by 70% and 80%, respectively, and was found to be more effective as an antioxidant than were α-tocopherol and curcumin.

Key words: red blood cells, turmeric, hemolysis, lipid peroxidation.

Introduction

Curcumins and aqueous extracts of turmeric (*Curcuma longa*) have been shown to possess inhibitory effects on lipid peroxidation (LPO).[1–3] Aqueous extracts of turmeric have been found to protect the lipid peroxide-induced DNA damage and Ca^{2+}-ATPase activity *in vitro*.[2,3] Administration of turmeric antioxidant protein (TAP) to CCl_4 fed animals protect tissues from peroxidation.[4] Normal canine erythrocytes are oxidized by hydrogen peroxide and hemolysis and lipid peroxidation of erythrocyte membranes has been observed.[5] The present study was carried out to investigate the protective effect of TAP on H_2O_2-induced red blood cell (RBC) LPO and hemolysis.

Methods

Preparation and isolation of TAP

Preparation and isolation of TAP from aqueous extract of turmeric was carried out as described by Selvam *et al.*[3] Turmeric tubers obtained from the local market were finely powdered. Approximately l.5 g of the powder was dissolved in 75 mL of boiling distilled water which was vortexed and centrifuged, and the clear supernatant collected.

The aqueous extract obtained was concentrated to a final volume of 10 ml by evaporation on a boiling water bath and was dialysed overnight against water at 25°C. The insoluble material was centrifuged out. Approximately one-fifth of the extract containing approximately 15 mg protein was then loaded onto a Sephadex-G-200 column (20 × 1.5 cm; Pharmacia, Piscataway, NJ, USA) previously equilibrated with Tris-HCI buffer (0.01 mol/L pH 7.4) and eluted with the same buffer. Fractions were collected and the A_{280} and antioxidant activity were measured.[3] Peak fractions containing maximum protein and maximum antioxidant activity were pooled and used for investigations.

Bood sampling

Blood was collected from healthy human volunteers into heparinized tubes (10 units/mL). Blood was then centrifuged at 2000 g for 7 min in a refrigerated centrifuge. Plasma and buffy coat were discarded. The RBC were washed three times with cold 0.15 mol/L saline. Washed RBC were used for investigation.

Hemolysis and lipid peroxidation

Hemolysis and LPO of erythrocytes were carried out using H_2O_2 by a modification of the method of Otomo and Fujisawa.[5] That is, 0.02 mol/L of normal human erythrocyte was taken in 1.0 mL of 0.02 mol/L phosphate buffered saline, pH 7.4, and mixed with 0.5 ml of the test sample (TAP, α-tocopherol and curcumin dissolved in ethanol) and 1.0 ml of H_2O_2 in phosphate buffered saline. In the absence of a test sample, 0.5 mL of buffer was included in the assay medium. The mixture was incubated for one hour in a shaking water bath at 37°C and then centrifuged at 1500 *g* for 10 min.

Following this, 1.5 mL of the red cell free supernatant solution from each tube was transferred to cuvettes and the optical density was read at 540 nm in a spectrophotometer using distilled water as a blank, which gives the extent of hemolysis.[6]

Lipid peroxidation was determined using 0.5 mL supernatant. To this, 1.0 mL of 5% trichloroacetic acid and 0.67% thiobarbituric acid were added and boiled in a water bath for 15 min. The tubes were cooled and centrifuged. Absorbance at 535 nm was determined.[7]

Inhibitory effects of the test compounds on hemolysis and LPO were estimated by the following equation where OD is optical density:

$$\text{Inhibitory ratio} = \frac{\text{OD with no addition of test compound} - \text{OD with addition of test compound}}{\text{OD with no addition of test compound}} \times 100$$

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Table 1. Effect of TAP, α-tocopherol and curcumin on the hemolysis and lipid peroxidation of human erythocytes induced by hydrogen peroxide

α-Tocopherol			Curcumin			TAP		
Concentration (µg)	LPO	Hemolysis	Concentration (µg)	LPO	Hemolysis	Concentration (µg)	LPO	Hemolysis
10	42.36	7.90	10	46.67	69.19	50	59.41	75.81
30	50.70	9.94	30	51.80	70.49	100	59.98	79.04
50	57.56	15.65	50	54.35	71.63	200	67.28	80.34
100	63.33	20.71	100	56.98	71.81	300	70.68	82.03

Values are averages of three experiments done in duplicate. Lipid peroxidation was measured in terms of TBARS and hemolysis in terms of the released hemoglobin. Values are given in terms of the per cent inhibitory ratio of the substance on lipid peroxidation (LPO) and hemolysis. See **Methods** for inhibitory ratio equation. TAP, turmeric antioxidant protein.

Results and discussion

The effect of TAP on the H_2O_2-induced hemolysis and LPO of human erythrocytes was studied in comparison with curcumin and α-tocopherol (lipophillic antioxidants). Data are presented in Table 1.

Lipid peroxidation was inhibited up to 63 and 57% at l00 µg concentration by α-tocopherol and curcumin, respectively, while TAP provided approximately 60% inhibition at 100 µg concentration and 70% inhibition at 300 µg concentration. Hemolysis of RBC was effectively protected by TAP. Turmeric antioxidant protein inhibited hemolysis by 80% whereas α-tocopherol could achieve only 20% inhibition of hemolysis.

Lipid peroxidation and hemolysis were inhibited by all of the three compounds studied with varying degrees of inhibition. Turmeric antioxidant protein was found to have a better inhibitory ratio than the other two compounds studied. Inhibition of LPO and hemolysis of mouse erythrocytes by curcuminoids (from turmeric) have also been reported.[1] H_2O_2-induced LPO has been shown to alter the human erythrocyte membrane bilayer which can be blocked by antioxidants such as vitamin E, butylated hydroxy toluene and butylated hydroxy anisole.[8] Turmeric antioxidant protein has also been shown to prevent inactivation of the membrane bound ATPase.[3]

Therefore, the protective effect of TAP may be due to its association with cell membranes, restoring enzyme activities which prevent membrane peroxidation and thereby RBC hemolysis. Thus TAP, a heat stable aqueous extracted antioxidant protein, may assume a role in relation to dietary consumption in Asia, although more needs to be known about whether an active peptide might be absorbed and physiologically active.

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References

1. Toda S, Miyase T, Arichi H, Taniwaza H, Takino Y. Natural antioxidants. III: Antioxidant components isolated from the rhizome of *Curcuma longa* L. Chem Pharm Bull 1985; 33: 1725.
2. Shalini VK, Srinivas L. Lipid peroxide induced DNA damage. Protection by turmeric (*Curcuma longa*). Mol Cell Biochem 1990; 95: 21.
3. Selvam R, Lalitha S, Gayathri R, Angayarkanni N. The antioxidant activity of turmeric (*Curcuma longa*). J Ethnopharmacol 1995; 47: 59.
4. Lalilta S, Selvam R. Prevention of CCl_4-induced hepatotoxicity by administration of aqueous extracted turmeric (*Curcuma longa*). Nutr Res 1999; 19: 429.
5. Otomo S, Fujisawa E. Stabilizing effects of anti-influmatory drugs on erythrocyte membrane. I: On the hemolysis and lipid peroxidation induced by H_2O_2 in canine erythrocytes. Yakugaku Zasshi 1970; 90: 1347.
6. Dacie JV. In: Hemolytic anemias, Part 1. The congenital anemias, 2nd edn. New York: Grune and Stratton Inc. 1960: 35.
7. Cyanamon HA, Isenburg JN, Ngryean CH. Erythrocyte malondialdehyde release *in vitro*: A functional measure of vitamin E status. Clin Chem Acta 1995; 15: 169.
8. Jain SK. The accumulation of malondialdehyde, a product of fatty acid peroxidation can disturb aminophospholipid organisation in the membrane bilayer of human erythrocytes. J Biol Chem 1984; 259: 3391.