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Note

Inhibition of radiation-induced lipid peroxidation by curcumin

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Abstract

The ability of curcumin, a natural antioxidant from turmeric, to inhibit radiation-induced lipid peroxidation in rat liver microsomes was examined. Curcumin was incorporated into microsomes during ultracentrifugation. The antioxidant has significant time- and concentration-dependent inhibitory effect on lipid peroxidation induced by τ-radiation. Inhibition of lipid peroxidation was also observed in microsomes samples previously saturated with N_2O. Curcumin also inhibited lipid peroxidation during the post-irradiation incubation. © 1997 Elsevier Science B.V.

Keywords: Curcumin; Lipid peroxidation; Antioxidant; τ-radiation

Curcumin (diferuloyl methane, CAS 458-37-7) possess many therapeutic properties including antiinflammatory and anticancer activities (Srimal, 1987). Our earlier studies have shown that curcumin is a powerful scavenger of superoxide anion (Kunchandy and Rao, 1990), hydroxyl radical (Kunchandy and Rao, 1989), nitrogen dioxide (Unnikrishnan and Rao, 1995), nitric oxide (Sreejayan and Rao, 1997) and the nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (Sreejayan and Rao, 1996). It also protects DNA against singlet oxygen-induced single strand break (Subramanian et al., 1994), and oxyhemoglobin from nitrite-induced oxidation (Unnikrishnan and Rao, 1992). We have also reported that curcumin is a potent inhibitor of iron-catalysed lipid peroxidation (Sreejayan and Rao, 1993, 1994).

The damaging effects of ionizing radiation in biological systems are at least partly mediated through oxygen free radicals (Riley, 1994). Curcumin has been shown to be effective in protecting chromosomal damage induced by τ-radiation in vivo (Abraham et al., 1993). In the present study we have investigated the effect of curcumin on radiation-induced lipid peroxidation.

Curcumin was synthesized by condensing vanillin with acetyl acetone as a boron complex (Pabon, 1964). The purity and chemical structure

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were confirmed by NMR, Mass and IR spectroscopy besides elemental analysis. 2-Thiobarbituric acid, tetraethoxypropane, butylated hydroxy toluene and trichloroacetic acid were purchased form Sigma Chemical Co. (St. Louis, MO). *tert*-Butanol was obtained from BDH Chemicals, England. Nitrous oxide used in the study was of Iolar grade from M/s Indian Oxygen Ltd. All other chemicals used were of analytical grade from reputed manufacturers. Steady state τ-radiolysis was carried out using a ^{60}Co τ-source (at Bhabha Atomic Research Centre, Bombay) the dose rate of which was found to be 630 Gy/h by following the standard Frickle method.

Microsomes from rat liver were isolated as reported earlier (Subramanian et al., 1993). For incorporating curcumin into the microsomes, 100 μl of 10 mM curcumin solution in ethanol was added to the microsomal pellet, homogenised, diluted to 11 ml with Buffer A and resedimented at 105 000 $\times$ *g* for 1 h. The concentration of curcumin in the microsomes was 223 μM. Appropriate control microsomes were prepared using 100 μl ethanol. Protein was estimated by the Lowry method (Lowry et al., 1951) and microsomes were resuspended at a concentration of 10 mg protein/ml in Buffer A, distributed as aliquots frozen in liquid nitrogen and stored at -20°C, for a maximum period of 2 weeks, until use.

For examining the protective effect of curcumin against lipid peroxidation induced by OH and other radical species generated by τ-radiolysis, microsomes were subjected to τ-radiation in 5 mM sodium phosphate buffer, pH 7.4. For irradiation, microsomal samples (final concentration 1.2 mg protein/ml) in iron-free 5 mM sodium phosphate buffer (pH 7.4) were placed in sealed tube in a Gamma cell (^{60}Co source). One set of samples were saturated with N_2O (by bubbling nitrous acid gas for 5 min prior to irradiation) while others were not. Post-irradiation incubation was carried out in air at 37°C in a shaker-water bath.

After incubations/irradiation, the extent of lipid peroxidation was estimated as thiobarbituric acid reactive substances (TBARS) in terms of malondialdehyde equivalents, using tetraethoxypropane as standard (Devasagayam et al., 1983).

Rat liver microsomes, when subjected to τ-irradiation of doses up to 630 Gy, resulted in lipid peroxidation (Fig. 1). The extent of peroxidation was dependent on the dose of the radiation. At an absorbed dose of 600 Gy dose about 6.9 nmol of TBARS/mg of microsomal protein were produced. When curcumin was incorporated into the microsomes, peroxidation was reduced to a significant extent. Owing to its poor solubility in aqueous buffers and since solvents such as ethanol and DMSO are themselves potent scavengers of hydroxyl radicals curcumin could not be added to the incubation mixture just prior to irradiation as is done in the conventional method. In order to overcome this, curcumin was incorporated into the microsomes during ultracentrifugation. Peroxidation of microsomes was radiation dose-dependent and at a radiation dose of 630 Gy, about 6.8 nmol TBARS/mg protein was formed (Fig. 1). Radiation-induced peroxidation of curcumin-incorporated microsomes was markedly less when compared to that observed with control microsomes. At 23 μM, curcumin inhibited 62% peroxidation induced by a radiation dose of 630 Gy (Fig. 1).

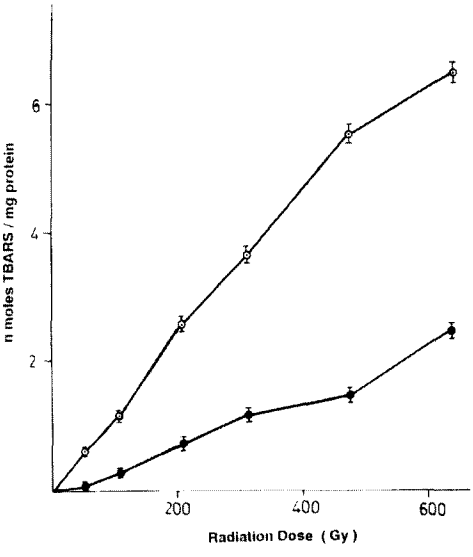

Fig. 1. Inhibition of radiation-induced lipid peroxidation in rat liver microsomes by curcumin. Values plotted represent mean $\pm$ S.E. of four independent experiments. (●) With, and (○) without curcumin.

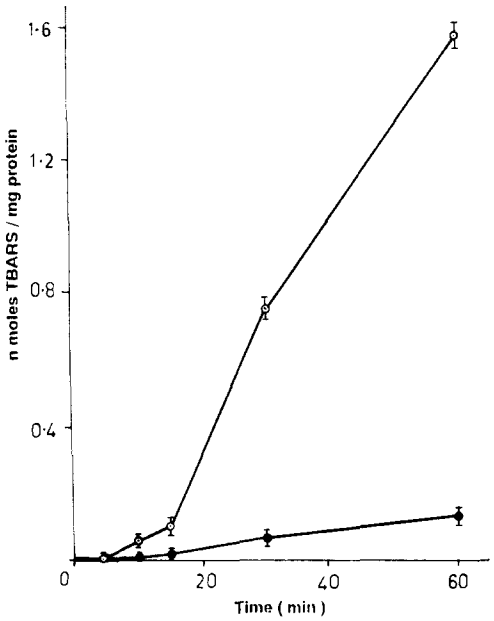

Fig. 2. Inhibition of post-irradiation lipid peroxidation in rat liver microsomes by curcumin in aerated buffer. Microsomes were exposed to 630 Gy dose of radiation from a ^{60}Co source and the values given are mean ± S.E. of four experiments. (●) In presence, or (○) absence of curcumin.

It has been reported that post-irradiation of lipids results in further increase in peroxidation. Hence, we incubated the microsomes for various lengths of time (at 37°C) following irradiation dose of 630 Gy. It resulted in a time-dependent increase in peroxidation (Fig. 2). About 1.6 nmol TBARS/mg protein was formed over a post-irradiation incubation period of 60 min. Curcumin effectively inhibited this post-irradiation peroxidation as well. The inhibition was time-dependent and was evident from the very beginning stages of incubation. At a concentration of 23 μM curcumin inhibited about 93% of the post-irradiation lipid peroxidation that had occurred during an incubation period of 60 min.

When the microsomes are irradiated in the presence of N_2O, the resulting lipid peroxidation is predominantly due to hydroxyl radicals. Radiation induced lipid peroxidation of rat liver microsomes that were previously saturated with nitrous oxide in Fig. 3. The lipid peroxidation observed in the control microsomes on irradiation following nitrous oxide saturation was marginally lesser than that observed without nitrous acid saturation. Since 10 mM *tert*-butanol, a known hydroxyl radical scavenger when included in the system resulted in 88% inhibition of the peroxidation observed (data not shown), hydroxyl radicals may be implicated as the key species responsible of lipid peroxidation in this model. At a radiation dose of 630 Gy, curcumin exhibited about 69% inhibition of microsomal lipid peroxidation in the nitrous oxide saturated system (Fig. 3). Data presented in Fig. 4 show the concentration-dependent inhibition by curcumin of lipid peroxidation in rat liver microsomes exposed to a radiation dose of 350 Gy both in the presence and absence of N_2O.

In conclusion, the results obtained in the present study indicate that curcumin is an effective inhibitor of lipid peroxidation induced by τ-radiation. This may explain in part the ability of curcumin to counter the radiation-induced damage.

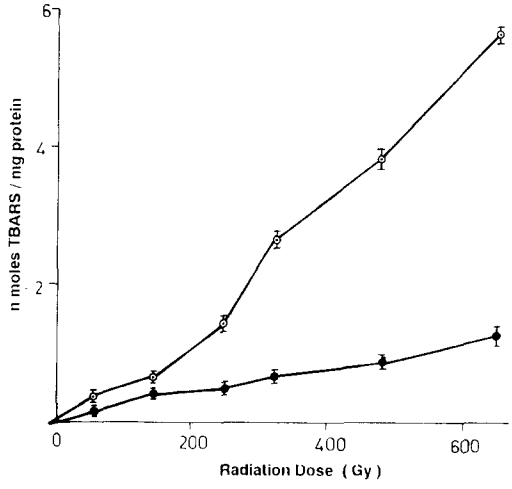

Fig. 3. Inhibition of radiation-induced lipid peroxidation by curcumin in N_2O saturated rat liver microsomes. Values plotted represent mean ± S.E. of four independent experiments. (●) With, and (○) without curcumin.

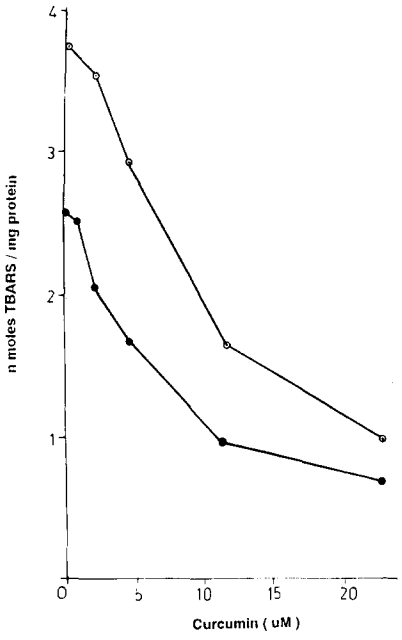

Fig. 4. Concentration-dependent effect of curcumin on the inhibition of radiation-induced lipid peroxidation in rat liver microsomes. Reaction mixtures containing microsomes were exposed to 350 Gy dose of radiation from a ^{60}Co source and the values given are mean ± S.E. of four experiments. (●) N_2O saturated, and (○) without N_2O saturation.

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