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Effect of Curcumin and Curcumin Copper Complex (1:1) on Radiation-induced Changes of Anti-oxidant Enzymes Levels in the Livers of Swiss Albino Mice

Prabhakar R. KOIRAM[1], Veeresh P. VEERAPUR[1], Amit KUNWAR[2], Beena MISHRA[2], Atanu BARIK[2], Indira K. PRIYADARSINI[2] and Unnikrishnan K. MAZHUVANCHERRY[1]*

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The effect of mononuclear copper (II) complex of curcumin in 1:1 stoichiometry (hereafter referred to as complex) administered 30 min before γ-irradiation (4.5 Gy) on alterations in antioxidant and Thiobarbituric acid reactive substances (TBARS) levels in livers was studied in comparison to curcumin at a dose of 50 mg/kg. The different antioxidants like GSH, GST, catalase, SOD, TBARS and total thiols were estimated in the liver homogenates excised at different time intervals (1, 2 and 4 h) post irradiation using colorimetric methods. There was a radiation-induced decrease in the levels of all the studied enzymes at 1 h post irradiation, while an increase was observed at later time points. Both curcumin and complex treatment in sham-irradiated mice decreased the levels of GSH and total thiols, whereas there was an increase in the levels of catalase, GST and SOD compared to normal control. Under the influence of irradiation, both curcumin and complex treatment protected the decline in the levels of GSH, GST, SOD, catalase and total thiols, and inhibited radiation-induced lipid peroxidation. Further, the complex was found to be more effective in protecting the enzymes at 1 h post irradiation compared to curcumin treated group. This may be due to the higher rate constants of the complex compared to curcumin for their reactions with various free radicals.

INTRODUCTION

Dietary antioxidants are known to strengthen the physiological antioxidant defense system either by scavenging reactive oxygen free radicals (ROS) or by protecting/elevating endogenous antioxidant enzymes. They also decrease the risk of many chronic diseases such as cancer and cardiovascular disorders.[1)] The antioxidant activity of curcumin, a natural polyphenol, found in the rhizomes of *Curcuma longa* (turmeric), exhibits anti-inflammatory, antineoplastic, antioxidant and chemopreventive activities.[2–4)] Curcumin and its analogues are known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free radical-mediated chain reaction leading to the damage of the cell membrane. Superoxide, an important member of the ROS, also participates in Haber-Weiss reaction and generates more damaging hydroxyl radicals.[5)] Superoxide dismuatase (SOD) is one of the most important antioxidant enzymes that catalyze superoxide neutralization by converting it to hydrogen peroxide and oxygen.[6,7)] SODs are being explored as useful pharmacological agents.[8,9)] The native enzyme, however, has several limitations, such as a short shelf life, low lipid solubility, and low penetration into cells.[7)] Several copper and manganese complexes of important compounds have been shown to efficiently catalyze superoxide dismutation.[10)] In this aspect, we had synthesized a mononuclear copper (II) complex of curcumin in 1:1 stoichiometry (hereafter referred to as complex). The complex has been evaluated for its ability to act as an SOD mimic and was found to exhibit free radical scavenging ability and inhibits γ-radiation-induced lipid peroxidation in model systems better than curcumin.[11)] These studies encouraged us to study the effect of the complex on time-dependent changes in liver antioxidant levels of irradiated Swiss albino mice.

*Corresponding author: Phone: +91820 2572195,
Fax: +91820 2570061,
E-mail: mkunnikrishnan@gmail.com
[1]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal – 576 104; [2]Radiation & Photochemistry Division, Bhabha Atomic Research Center, Mumbai - 400 085.
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MATERIALS AND METHODS

Chemicals

Curcumin, 5,5'-dithio-bis-2-nitrobenzoic acid [DTNB, Ellman's reagent], 1-chloro-2, 4-dinitrobenzene (CDNB), glutathione (GSH), superoxide dismutase (SOD) were from Sigma chemical Co, USA, All the other chemicals used were of analytical grade procured from Qualigens Fine chemicals, Mumbai, India. The complex was synthesized, purified and characterized as described in our earlier publication.[11)]

Animals

All experiments were carried out on inbred Swiss albino mice (both male and female) from the animal colony of Kasturba Medical College, Manipal that were 8–10 weeks old and weighed 25 ± 5 g. The colony was maintained under controlled conditions of temperature (23 ± 2°C) and humidity (50 ± 5%) and a 12-h light–dark cycle. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard mouse food and water. All studies conducted were in compliance with the prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by Institutional animal ethical committee.

Irradiation

Mice were placed in well-ventilated perspex boxes (23.5 × 23.5 × 3.5 cm, partitioned into 3 × 3 × 11-cm cells for each animal) and exposed to whole-body γ-radiation from a ^{60}Co Theratron Teletherapy unit (Siemens, Germany) at a dose rate of 1.66 Gy/min and a source-to-surface distance (SSD) of 64.2 cm. The Department of Radiation Oncology and Radiation Physics, Kasturba Medical College, Manipal, helped with radiation dosimetry calculations.

Experimental design

Animals were divided into groups of five each and treated as follows:

- 0.3 ml of 0.5% Carboxy methylcellulose (CMC) per mouse, 30 min prior to sham-irradiation (Normal control).
- 0.3 ml of 0.5% CMC per mouse, 30 min prior to γ-irradiation (Radiation Control).
- 50 mg/kg of curcumin or complex, 30 min prior to γ-irradiation.
- 50 mg/kg of curcumin or complex, 30 min prior to sham-irradiation.

Animals were sacrificed by cervical dislocation at different time intervals (1, 2 and 4 h) post irradiation and livers were excised and antioxidant enzyme activities were estimated.

Estimation of antioxidant enzyme activities

A 10% homogenate of the excised livers of different groups were prepared and protein content was determined.[12)] The homogenate was further subjected to the estimation of GSH, catalase, GST, SOD and total thiols using standardized protocols of our laboratory quoted in our previous publications.[13)] Lipid peroxidation levels of the liver homogenates were also determined.[14)]

Statistical evaluation

The data are expressed as mean ± S.E.M. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-test using GraphPad Prism version 4.00.

RESULTS

Glutathione (GSH)

In comparison to normal control group (**16.44 ± 5.18 μmoles/ mg of protein**), curcumin and complex treatment resulted in decrease of GSH levels in sham-irradiated mice at 1 and 2 h followed by an increase at 4 h. Radiation control group also showed decreased GSH levels at 1 h, which increased at 2 h and 4 h compared to normal control mice. Both curcumin and complex treated irradiated groups showed increased GSH levels at all the observed intervals compared to radiation control. Complex showed increased GSH levels at 1 h post irradiation compared to curcumin treated group [Fig. 1A].

Glutathione-S-transferase

Sham-irradiated group treated with curcumin showed a three fold increase in GST after 1 h and a 2-fold increase at 4 h compared to normal control mice (**0.013 ± 0.0039 U/ mg of protein**). Complex also showed induction of GST similar to that of curcumin. Radiation-treatment decreased the levels of GST at all the observed intervals compared to normal control mice except at 2 h. Under the influence of radiation, complex maintained the GST levels where as curcumin decreased GST levels at 1 h and at further time points maintained to near normal levels. However complex treated mice were found to have higher GST levels at 1 h post irradiation compared to curcumin treated group [Fig. 1B].

Catalase

Administration of curcumin or complex resulted in the elevation of catalase activity at 4 h compared to normal control mice (**48.56 ± 7.37 U/ mg of protein**). Radiation-control showed decrease in catalase activity at 1 h and 2 h but an increase was observed at 4 h compared to normal control mice. Both curcumin and complex treated groups under the influence of radiation, protected mice from a fall in catalase activity at all observed intervals, compared to the radiation control. Catalase levels were found to be higher at 1h in complex treated group compared to curcumin treatment [Fig. 1C].

Superoxide dismutase

Curcumin and complex treatment in sham-irradiated mice, increased the SOD levels at all the observed intervals, compared to the normal control mice (**16.32 ± 2.14 U/ mg of protein**). Radiation control group also showed marginal increase in SOD levels at 1 h, followed by 2-fold increase at

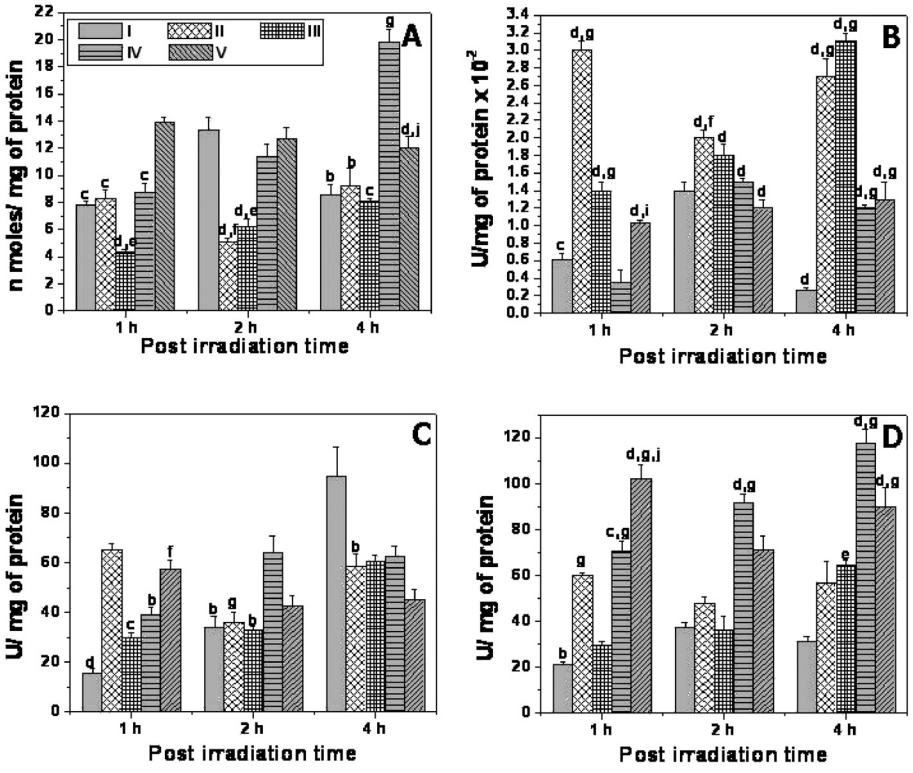

Fig. 1. Effect of 50 mg/kg each of curcumin and complex on liver antioxidant enzymes. **(A);** GSH [GSH in normal control mice = 16.44 ± 5.18]. **(B);** GST [GST in normal control mice = 0.013 ± 0.0039] **(C);** Catalase [catalase in normal control mice = 48.56 ± 7.37]. **(D);** SOD [SOD in normal control mice = 16.32 ± 2.14]. I-Radiation control; II-Curcumin + sham-irradiation; III- Complex + sham-irradiation; IV-Curcumin + 4.5 Gy irradiation; V- Complex + 4.5 Gy irradiation. Values are mean ± SEM (n = 5) (b) $p < 0.05$, (c) $p < 0.01$, (d) $p < 0.001$ compared to normal control mice; (e) $p < 0.05$, (f) $p < 0.01$, (g) $p < 0.001$ compared to radiation control at corresponding time interval. (i) $p < 0.01$, (j) $p < 0.001$ complex compared to curcumin.

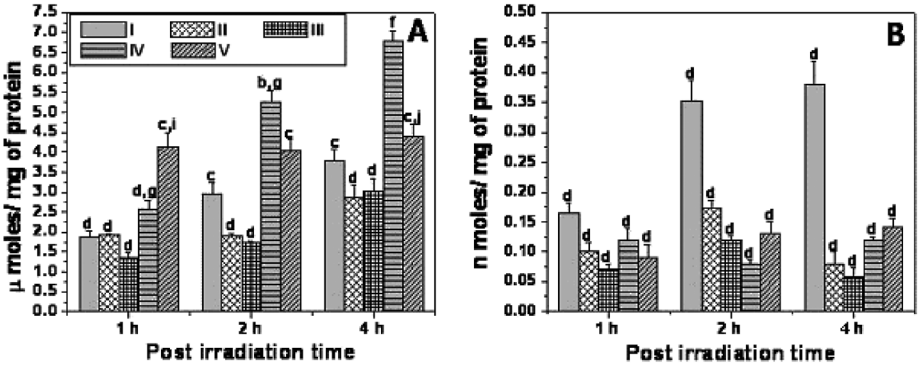

Fig. 2. Effect of 50 mg/kg each of curcumin and complex on liver non-enzymatic antioxidant levels. **(A);** Total thiols [Total thiols in normal control mice = 7.81 ± 1.36]. **(B);** TBARS [TBARS in normal control mice = 0.086 ± 0.066]. I-Radiation control; II-Curcumin + sham-irradiation; III- Complex + sham-irradiation; IV-Curcumin + 4.5 Gy irradiation; V- Complex + 4.5 Gy irradiation. Values are mean ± SEM (n = 5) (b) $p < 0.05$, (c) $p < 0.01$, (d) $p < 0.001$ compared to normal control mice; (f) $p < 0.01$, (g) $p < 0.001$ compared to radiation control at corresponding time interval. (j) $p < 0.001$ Cu (II)-curcumin complex compared to curcumin.

2 h and 4 h compared to normal control mice. Under the influence of radiation, both curcumin and complex increased the SOD activity at all the observed intervals compared to radiation control. However, the complex treated group showed a significant ($p < 0.01$) increase in the activity at 1 h in comparison to the curcumin treated group [Fig. 1D].

Total Thiols

Curcumin and complex treatment in sham-irradiated group, decreased total thiol levels significantly ($p < 0.001$) at all observed time intervals compared to normal control mice (**7.81 ± 1.36 μmoles/ mg of protein**). Radiation control also showed significant decrease in thiol levels ($p < 0.001$) at all time intervals compared to normal control mice. Curcumin treated irradiated mice showed about 2-fold ($p < 0.001$) increase in thiols at all observed time intervals in comparison to radiation control. Thiol levels were significantly ($p < 0.01$) higher in complex treated group at 1 hr post irradiation compared to curcumin treated group. However curcumin maintained heightened thiol levels at all other time points [Fig. 2A].

Lipid peroxidation (LPX)

Radiation-treatment increased the LPX at all the observed time intervals compared to the normal control mice (**0.086 ± 0.066 nmoles/ mg of protein**). Curcumin and complex treated irradiated groups reduced the LPX levels at all the studied time intervals compared to the radiation control. Further, complex treated mice showed less TBARS after 1 h post irradiation compared to curcumin treated mice [Fig. 2B]

DISCUSSION

Most of the toxic effects of ionizing radiations to normal tissue are due to the generation of ROS by the radiolysis of water, which triggers formation of several reactive intermediates. To overcome such events, living cells are equipped with comprehensive and integrated endogenous enzymatic and non-enzymatic antioxidant systems such as GSH, vitamin E, ascorbate, β-catotene [non-enzymatic antioxidants] and Cu-Zn and MnSODs, catalase, GST and GPx [enzymatic antioxidants].[15)]

Free radicals generated by irradiation also react with unsaturated lipids generating hydroperoxides, which in turn can induce changes in the lipid bilayer thereby altering the membrane permeability and inducing lipid peroxidation.[16)] Curcumin and complex both reduced the radiation-induced lipid peroxidation in liver. Complex treated mice showed less TBARS compared to curcumin treated group after 1 h of irradiation. This is further supported by our earlier *in-vitro* comparative studies, where we found complex (IC_{50} = 2.5 μM) more effective in reducing the lipid peroxidation in phosphatidyl choline liposomes compared to curcumin (IC_{50} = 4.0 μM).[11)]

The depletion in GSH after exposure to γ-radiation may be due to the reaction of GSH with free radicals resulting in the formation of thiyl radicals that associate to produce GSSG.[17)] Further, normal synthesis/repair of GSH will be impaired due to damage to DNA and membranes. Total thiols are the other important sources of -SH- groups in enzyme formation and DNA synthesis.[17)] Radiation toxicity denatures proteins and causes conformational changes, which render them inactive. Curcumin and complex treated sham-irradiated group showed significantly decreased GSH and total thiol levels, possibly because of the non-enzymatic Michael-type addition reaction of curcumin with GSH/total thiols or enzymatic conjugation with GSH in the presence of GST.[18)] However the levels of GSH were found to be restored with increase in time after drug administration. Under the influence of radiation, both the tested agents were found to increase the levels of GSH and thiols at all the observed time intervals.

GST is a phase II metabolic enzyme involved in detoxification. Curcumin and complex treatment induced GST by 2–3 folds in sham-irradiated group at all the observed time intervals. Such an induction of GST in various organs is also reported earlier from our group. However, the exact mechanism of this hepatic induction is not yet known.[19)] Radiation-treatment decreased the levels of GST at all the observed time intervals while curcumin and complex treatment protected against this decline. Further, complex was found to be more effective than curcumin at 1 h post irradiation.

SOD and catalase are other important enzymes, which protect against the free radical injury mediated by $O_2^{\bullet-}$ and H_2O_2. Curcumin and complex treatment increased the levels of these enzymes at all the observed time intervals in irradiated mice. This may be explained by the fact that both complex and curcumin reacts with $O_2^{\bullet-}$ with a bimolecular rate constants of $1.97 \pm 0.30 \times 10^5$ M^{-1} s^{-1} and 4.6×10^2 M^{-1} s^{-1} respectively in DMSO.[11)] The better protection at 1 h post irradiation, offered by the complex in comparison to curcumin, may be explained due to its higher rate constants with various free radicals, as reported previously.[11)]

In conclusion, complex affords better protection than curcumin in preventing the depletion of the antioxidant enzymes caused by radiation, at one hour post irradiation.

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