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Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs

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ABSTRACT

The present study was conducted to investigate the antioxidative effect of curcumin against sodium arsenite-induced oxidative damage in rat. Animals were divided into four groups, the first group was used as control. Groups 2, 3 and 4 were orally treated with curcumin (15 mg/kg BW), sodium arsenite (Sa, 5 mg/kg BW) and sodium arsenite plus curcumin, respectively. Rats were orally administered their respective doses daily for 30 days. Results showed that Sa increased thiobarbituric acid-reactive substances (TBARS) in plasma, liver, kidney, lung, testes and brain. While, the activities of glutathione S-transferase, superoxide dismutase and catalase and the content of sulfhydryl groups (SH-groups) were significantly decreased in plasma and tissues compared to control. Treatment with curcumin alone reduced the levels of TBARS, while induced the activities of the antioxidant enzymes, and the levels of SH-groups. The presence of curcumin with Sa reduced the induction in the levels of TBARS and induced the decrease in the activities of antioxidant enzymes and the levels of SH-groups. Results indicated that treatment with Sa decreased body weight and increased liver weight compared to control. The presence of curcumin with Sa alleviated its toxic effects. It can be concluded that curcumin has beneficial influences and could be able to antagonize Sa toxicity.

1. Introduction

Arsenic being a potent environmental toxic agent, leads to development of various hazardous effects on human health. All human populations are exposed to arsenic and its compounds through occupational or environmental processes (Ramanathan et al., 2003). More than 80% of arsenic compounds are used to manufacture products like glass and semiconductors, dyestuffs and as an additive to metal alloys. Also, it is used in agricultural applications such as insecticides, herbicides, fungicides, algaecides, sheep dips and wood preservatives (Tchounwou et al., 2002). Human exposure to arsenic is associated with cancer, organ injury and immunotoxicity (Patrick, 2003). There is an evidence suggesting that arsenic toxicity involves oxidative damage (Izquierdo-Vega et al., 2006), mainly by the interaction of arsenic with protein thiols that are central components of redox-sensitive proteins in redox signaling and control pathways (Hansen et al. 2006). Several studies have demonstrated that liver is the primary arsenic metabolizing organ (Hughes et al., 2003). Metabolic conversion of arsenic into methylated products is a multistep process that yields mono–di and trimethylated arsenic forms that have different toxic potential than that of the parent compound arsenic. Many biological processes have been identified as involved in arsenic-induced toxicity and carcinogenicity. These include induction of micronuclei, alterations in gene expression, induction of oxidative stress, alteration in enzyme activities, change in carbohydrate metabolism, inhibition of DNA repair, perturbation of DNA methylation, alteration of signal transduction pathways, altered cell cycle control, aberrant differentiation, and altered apoptosis (Kitchin, 2001; Manna et al., 2007; Bagnyukova et al., 2007).

Unbound inorganic arsenic generates reactive oxygen species (ROS) during redox cycling and metabolic activation processes (Bashir et al., 2006). Various studies reported that arsenic could participate in the cellular oxidation–reduction reactions resulting with the formation of excess ROS such as superoxide anion (O_2^-) and hydroxyl radical (OH**.**) via a chain reaction (Liu et al., 2001; Garcia-Shavez et al., 2006) causing oxidative stress. Toxic effects of arsenic are mediated primarily by triggering the production of reactive oxygen species (ROS), inhibiting the activity of enzymes like superoxide dismutase and catalase, leading to alterations in

Abbreviations: Sa, sodium arsenite; TBARS, thiobarbituric acid-reactive substances; TBA, thiobarbituric acid; GST, glutathione S-transferase; SOD, superoxide dismutase; CAT, Catalase; SH-groups, sulfhydryl groups; GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; GSH-Px, glutathione peroxidase; GR, glutathione reductase; NO, nitrogen oxide radical; LPO, lipid peroxidation.

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cells' intrinsic antioxidant defenses; and resulting in oxidative stress or disturbed antioxidant/pro-oxidant ratio (Liu et al., 2001).

Endogenous antioxidants, including vitamins, trace minerals, antioxidant enzymes, tripeptides and reductants, may quench reactive oxygen species (ROS) or suppress lipid peroxidation. Curcumin, a yellow orange dye derived from the rhizomes of *Curcuma longa* turmeric, which is used as a spice and food-coloring agent. Curcumin has been shown to have a broad spectrum of biological activities such as anti-inflammatory, anti-neoplastic, antimutagenic and antioxidant (Naik et al., 2004). Research has shown curcumin to be a powerful scavenger of the superoxide anion, the hydroxyl radical and nitrogen dioxide (Daniel et al., 2004). The interaction of curcumin with sodium arsenite has not been referred yet. Therefore, the present study aimed to investigate the protective role of curcumin against sodium arsenite-induced toxicity in plasma and organs of male rats.

2. Materials and methods

2.1. Chemicals

Sodium arsenite ($NaAsO_2$) was purchased from Sigma Chemical Company, St Louis, MO, USA, while curcumin was purchased from Superior Nutrition and Formulation by Jarrow Formulas, Los Angeles, CA90035-4317. All other chemicals used in the experiment were of analytical grade.

2.2. Experimental design

Twenty eight male Sprague–Dawley rats weighting 200–235 g were used in the present experiment. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of seven and given food and water *ad libitum*. After two weeks of acclimation, animals were divided into four equal groups. The first group was used as control. Groups 2, 3 and 4 were orally treated with curcumin (15 mg/kg BW), sodium arsenite (Sa, NaAsO2, 5 mg/kg BW) and sodium arsenite (5 mg/kg BW) plus curcumin (15 mg/kg BW), respectively. Rats were orally administered their respective doses every day for 30 days. At the end of the experiment, body weights of rats were recorded. Animals were sacrificed by decapitation and brain, liver, lung, kidney and testes were immediately removed and weighed then the organs weight ratio was calculated. The relative weight of organs (%) was calculated as g/100 g body weight.

2.3. Plasma collection

Trunk blood samples were collected from the sacrificed animals and placed immediately on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 860*g* for 20 min and stored at −60 °C. Stored plasma samples were analyzed for antioxidant enzymes, TBARS and SH-group.

2.4. Tissue preparation

Liver, kidney, lung, testes and brain were immediately removed; weighed and washed using chilled saline solution. Tissues were minced and homogenized (10% w/v), separately, in ice-cold 1.15% KCl-0.01 M sodium, potassium phosphate buffer (pH 7.4) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 *g* for 20 min at 4 °C, and the resultant supernatant was used for the determination of antioxidant enzyme assays and TBARS, total SH groups and protein content.

2.5. Determination of Glutathione S-transferase (GST) activity in plasma and tissue

Glutathione S-transferase (GST; EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig et al. (1974). P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 310 nm using UV-Double Beam spectrophotometer.

2.6. Determination of catalase (CAT) activity in plasma and tissue

The enzyme catalase (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity in plasma and tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Aebi, 1984).

2.7. Determination of super oxide dismutase (SOD) activity in plasma and tissue

Super oxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. Epinephrine was added to the assay mixture, containing tissue supernatant and the change in extinction coefficient was followed at 480 nm in a Spectrophotometer.

2.8. Tissue and plasma thiobarbituric acid-reactive substances assay

According to the method of Esterbauer and Cheeseman (1990), the extent of lipid peroxidation in terms of thiobarbituric acid-reactive substances (TBARS) formation was measured. Plasma and tissue supernatant was mixed with 1 ml TCA (20%), 2 ml TBA (0.67%) and heated for 1 h at 100 °C. After cooling, the precipitate was removed by centrifugation. The absorbance of the sample was measured at 535 nm using a blank containing all the reagents except the sample. As 99% TBARS are malondialdehyde (MDA), so TBARS concentrations of the samples were calculated using the extinction co-efficient of MDA, which is $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.9. Estimation of total sulfhydryl group in plasma and tissue

The determination of total tissue sulfhydryl group was carried out according to the method of Ellman (1959) with minor modification. Tissue supernatant was taken up and 10 mM – DTNB (5,5′-dithio-bis-2-nitrobenzoic acid), pH 7.0, prepared in 10 mM – phosphate buffer (pH 7.0) was added. The mixture was kept at room temperature for 20 min and the absorbance was measured at 412 nm.

2.10. Protein estimation

The protein content of the tissue homogenates mentioned earlier was determined by following the method described by Lowry et al. (1951) using bovine serum albumin as a standard.

2.11. Statistical analysis

Data were analyzed according to Steel and Torrie (1981). Statistical significance of the difference in values of control and treated animals was calculated by (*F*) test at 5% significance level. Data of the present study were statistically analyzed by using Duncan's Multiple Range Test (SAS, 1986).

3. Results

3.1. Effect of curcumin on sodium arsenite-induced changes in antioxidant enzyme activity in plasma and organs

Tables 1 and 2 show that treatment with Sa caused a significant ($P < 0.05$) decrease in the activities of GST, SOD and CAT in plasma and different organs. Treatment with curcumin alone caused significant ($P < 0.05$) increase in the activities of GST, SOD and CAT compared to both Sa-treated rats and control. In addition, a significant recovery relating to GST, SOD and CAT was observed in response to the presence of curcumin with Sa.

3.2. Effect of curcumin on sodium arsenite-induced changes in plasma and tissue lipid peroxidation

Thiobarbituric acid assay (TBA) was used to measure the extent of lipid peroxidation induced by Sa in plasma and different organs of rats. Results indicated that TBARS level was significantly ($P < 0.05$) increased in plasma, liver, testes, brain, kidney and lung of rats treated with Sa (Tables 3 and 4). Treatment with curcumin alone caused significant ($P < 0.05$) decrease in plasma and organs TBARS compared to both Sa-treated rats and control. In addition, the presence of curcumin with Sa reduced the elevation in plasma and tissue TBARS to control values.

3.3. Effect of curcumin on sodium arsenite-induced changes in plasma and tissue total sulfhydryl group and protein content

The content of SH-group was significantly ($P < 0.05$) decreased in plasma and different organs of rats treated with Sa compared