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Original article

Anti-oxidative effects of curcumin on immobilization-induced oxidative stress in rat brain, liver and kidney

Saeed Samarghandian[a,*], Mohsen Azimi-Nezhad[b], Tahereh Farkhondeh[c], Fariborz Samini[d]

[a] Department of Basic Medical Sciences, Neyshabur University of Medical Sciences, Neyshabur, Iran
[b] Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Department of Immunogenetics, BuAli Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[d] Department of Neurosurgery, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Restraint stress has been indicated to induce oxidative damage in tissues. Several investigations have reported that curcumin (CUR) may have a protective effect against oxidative stress. The present study was designed to investigate the protective effects of CUR on restraint stress induced oxidative stress damage in the brain, liver and kidneys. For chronic restraint stress, rats were kept in the restrainers for 1 h every day, for 21 consecutive days. The animals received systemic administrations of CUR daily for 21 days. In order to evaluate the changes of the oxidative stress parameters following restraint stress, the levels of malondialdehyde (MDA), reduced glutathione (GSH), as well as antioxidant enzyme activities superoxide dismutase (SOD) glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) were measured in the brain, liver and kidney of rats after the end of restraint stress. The restraint stress significantly increased MDA level, but decreased the level of GSH and activists of SOD, GPx, GR, and CAT the brain, liver and kidney of rats in comparison to the normal rats ($P < 0.001$). Intraperitoneal administration of CUR significantly attenuated oxidative stress and lipid peroxidation, prevented apoptosis, and increased antioxidant defense mechanism activity in the tissues versus the control group ($P < 0.05$). This study shows that CUR can prevent restraint stress–induced oxidative damage in the brain, liver and kidney of rats and propose that CUR may be useful agents against oxidative stress in the tissues.

1. Introduction

Stress is a physiological condition induced by adverse stimuli that imbalance the physiological body function [1]. In such situations, body cells need to utilize high energy for acclimatizing to abnormal environmental conditions such as an increase in respiration rate [1]. However, increased metabolic rate also causes free radicals over-production [1]. Excess free radicals cause oxidative damage to cellular biomolecules including proteins, lipids and nucleic acids in various tissues [2]. Restraint stress is one of the classic techniques to induce physiological and psychological stress, which changes the activities of antioxidant enzymes [3]. Oxidative stress is supposed to be a main factor in the progress of many different disorders such as neurodegenerative diseases, chronic kidney disease, hepatic inflammation, hypercholesterolemia, diabetes, hepatic cirrhosis, etc. [4]. Therefore, the morbidity rate to various disorders may be increased with long-term exposure to physiological or psychological stress [3]. A number of natural agents have been shown to exhibit therapeutic effects against neurodegenerative diseases [5]. Curcumin (CUR), the active component in the turmeric, the powdered rhizome of *Curcuma longa* Linn, has a wide range of biological activities, such as antioxidant, antiinflammatory, antiepileptic, analgesic, chemotherapeutic and antibacterial effects [6,7]. Furthermore, it has been used because of its thrombosuppressive, cardio-protective, antiarthritic, hepatoprotective, hypoglycemic, renoprotective, neuroprotective, antineoplasic and antiproliferative effects [8–10].

CUR has strong antioxidant activity [11]. This substance exerts antioxidant effect by affecting on reactive species, scavenging superoxide anion (O^-), peroxynitrite (NOO), nitric oxide (NO), peroxyl radicals (ROO) and hydroxyl (OH^-) radicals and inducing

* Corresponding author.
E-mail address: samarghandians@mums.ac.ir (S. Samarghandian).

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an up-regulation of antioxidant proteins [11]. Phenolic groups of CUR are responsible for its ability to react with reactive species and might likely be one of the mechanisms via which CUR administration protects cells from oxidative damage [11]. CUR is able to indirectly induce the expression of antioxidant proteins such as superoxide dismutase (SOD), catalase(CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), γ-glutamyl cysteine ligase (γGCL) [11]. Furthermore, it has been illustrated that CUR can enhance the synthesis and level of reduced glutathione (GSH) by induction of γGCL [11]. However, previous studies have not reported that CUR may prevent chronic stress induced tissue damages via protection against oxidative stress.

Strong evidences have indicated the effect of various antioxidants on the chronic restraint, or immobilization-induced stress model [12]. Therefore, the present study was designed to investigate the effect of CUR on oxidative stress-related changes in the brain, liver and kidney of restraint stress.

2. Materials and methods

2.1. Reagents

All purified enzymes, coenzymes, substrates, standards, buffers, kits and the other chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, USA) and the corticostrone ELIZA kit were purchased from Cusabio (Cusabio Biotech Co., Ltd).

2.2. Animals

Wistar albino rats (2 months; 200 ± 13 g) were bred at the university experimental animal care center. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water.

2.3. Study design

Rats were randomly divided into eight experimental groups (8 rats per group) as follows: 1) Vehicle (Veh) + no-stress (NS) (Veh-NS); 2) Vehicle + stress (Veh-S), 3) CUR (10 mg/kg, IP) + no-stress (CUR10-NS), 4) CUR (20 mg/kg, IP) + no-stress (CUR20-NS); 5) CUR (30 mg/kg, IP) + no-stress (CUR30-NS), 6) CUR (10 mg/kg, IP) + stress (CUR10-S), 7) CUR (20 mg/kg, IP) + stress (CUR20-S), 8) CUR (30 mg/kg, IP) + stress (CUR30-S). Restraint stress was performed using a rodent restrainer made of plexiglas that closely fit to the rats' body [13]. For chronic restraint stress, rats were kept in the restrainers for 1 h per day for 21 consecutive days. The animals received systemic administrations of vehicle (0.1% dimethyl sulfoxide-DMSO, 1 ml, i.p.) and CUR daily for 21 days [13]. At the end of the experimental period, animals were anesthetized with ether and blood was subsequently collected from the retro orbital sinus. Blood and sera were separated by centrifugation at 5000 RPM for 5 min for corticosterone measurement. Then, after decapitation brain, liver and kidney were removed for measuring the oxidative stress markers.

After the removal of tissues, they were washed in cold 0.9% saline and kept at −70 °C until used for preparation of homogenates with a homogenizer. Each tissue was finely minced and homogenized in 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 × *g* for 15 min at 4 °C (Beckman refrigerated, Ultracentrifuge). The homogenate and supernatant were used for the assays.

2.4. Corticosterone evaluation

Under deep anesthesia, blood was collected from the retro orbital sinus of rats. Blood was allowed to clot and sera were separated using centrifugation at 5000 RPM for 5 min and stored at −80 °C until use. Total serum level of corticosterone was measured by ELISA kits (CORT ELISA Kit CSB-E07014r).

2.5. Measurement of lipid peroxidation

MDA results from degradation of polyunsaturated lipids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substances (TBARS) to form a 1:2 MDA-TBA adduct, which absorbs at 532 nm. Thus, the quantity of TBARS is proportionate to the amount of MDA. Concentration of TBARS is determined according to a method of Mihara and Uchiyama. The concentration of TBARS was calculated using MDA standard curve and was expressed as nmol/mg of protein [14].

2.6. Estimation of GSH

GSH was measured by the method of Beutler et al. (1963). Briefly, to 0.1 ml of sample was added 0.9 ml distilled water and 1.5 ml of precipitating reagent (3.34 g *meta*-phosphoric acid, 0.4 g EDTA and 60.0 g sodium chloride). Tubes were shaken and allowed to stand for 5 min at room temperature (25 ± 1 °C). The mixture was centrifuged for 15 min at 4000 RPM at 4 °C. In 1.0 ml supernatant, 4.0 ml of phosphate solution (0.3 M disodium hydrogen phosphate) and 0.5 ml 5-50-dithiobis-(2-nitrobenzoic acid) (DTNB) (80 mg in 1% sodium citrate) were added. The development of yellow color complex was read immediately at 412 nm on a spectrophotometer. A standard curve using GSH was prepared and GSH concentration in the experimental samples was extrapolated from the standard curve. GSH concentration was calculated and expressed as μmol of GSH/mg protein [15].

2.7. Measurements of enzymes

The activity of SOD was determined by the method of (Marklund and Marklund 1979), using inhibition of pyrogallol autoxidation at pH8.The specific activity of SOD is expressed as units per mg protein per minute [16]. The activity of GPx was measured by the method of Paglia and Valentine [17]. GPx catalysis the oxidation of Glutathione by Cumene Hydroperoxide. In the presence of GR and NADPH the oxidized Glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm is measured. GR catalyses the reduction of glutathione in the presence of NADPH, which is oxidized to NADP. The decrease in absorbance at 340 nm is measured. The levels of GPx and GR were expressed as U/mg protein. CAT activity was assayed by H_2O_2 consumption, following Aebi's (Aebi 1984) method and modified by Pieper et al. (1995) [18,19].

2.8. Protein estimation

Protein was estimated in subcellular fractions by the method of Bradford (1976) using bovine serum albumin (BSA) as standard [20].

2.9. Statistical analysis

All experiments were carried out in triplicate. Each group consisted of eight rats. One way analysis of variance (ANOVA) was performed and Tukey post hoc test was used for multiple comparisons. Statistical analyses were performed using the InStat 3.0 program. The results are expressed as mean ± SEM. The results originated from analysis of tissues and serum. Linear correlation