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Immunomodulatory effects of nanocurcumin in arsenic-exposed rats

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ABSTRACT

We evaluated whether the nanoformulation of curcumin could be more effective than free curcumin against arsenic-induced immune dysfunction in rats. Curcumin was encapsulated in polylactic-co-glycolic acid (PLGA). Nanocurcumin (CUR-NP) exhibited a spherical shape with the mean particle size of 130.8 nm. Rats were randomly divided into five groups of six each. Group I was kept as the control. In Group II, rats were exposed to sodium arsenite (25 ppm) daily through drinking water for 42 days. Groups III, IV and V were treated with arsenic as in Group II, however, they were administered with nanoparticle, curcumin (100 mg/kg bw) and CUR-NP (100 mg/kg bw), respectively, by oral gavage during the last 14 days of arsenic exposure. At term, serum and spleen were collected. Immune dysfunction was evaluated by assessing cellular and humoral immunities. Arsenic significantly decreased the splenic lymphocyte proliferation in response to the antigen — Keyhole Limpet Hemocyanin (KLH) and mitogen — concanavalin-A. Arsenic reduced both the delayed type hypersensitivity response and secondary antibody (IgG) response to KLH. It also reduced the lipopolysaccharide-stimulated nitric oxide production in splenic lymphocytes. Free curcumin and CUR-NP treatment significantly attenuated these arsenic-mediated effects. However, the magnitude of the effects indicates that CUR-NP has better ameliorative potential than free curcumin at the equivalent dose level.

1. Introduction

Contamination of drinking water with arsenic is a burning global public health issue. Arsenic concentrations much higher than the maximum permissible level (10 ppb) have been reported in groundwaters in several countries, particularly in the Indian subcontinent. In fact, in West Bengal (India) and Bangladesh the situation has been designated as the greatest arsenic calamity to mankind [1]. Various studies document that chronic exposure to arsenic through drinking water causes a wide range of adverse effects, including cancer of skin and epithelial tissues, diabetes, vascular diseases, and neuropathy [2–4]. Further, arsenic can have significant effects in the immune system, including inhibition of contact hypersensitivity response [5], suppression of secondary antibody (IgG) response to T-cell-dependent antigen [6], suppression of human peripheral lymphocyte activation and response [7] and altered expression of cytokines [8,9]. These changes can alter the immune function in the exposed populations. However, it's a matter of concern that there is no effective remedy for arsenic-induced immune dysfunction without discernable demerits.

Curcumin ((1E,6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a yellow colored polyphenol powder obtained from *Curcuma Longa*. It possesses low intrinsic toxicity and a wide range of pharmacological activities that include antioxidant, antitumor, anti-inflammatory and immunomodulatory properties [10–13]. Despite the considerable promise that curcumin is an efficacious and safe compound for multiple medicinal benefits, there are some demerits with respect to the therapeutic effectiveness of curcumin, viz., reduced bioavailability of orally administered curcumin, fast metabolism by the liver and rapid systemic elimination [14]. To overcome this obstacle, different approaches, including incorporation of curcumin into liposomes and complexation with phospholipids are being studied [15]. However, these did not satisfactorily improve the therapeutic effectiveness [16]. More recently, the approach of biodegradable polymer nanoparticles has been developed [17,18]. This offers promise for therapeutic effectiveness of curcumin by increasing its bioavailability, solubility and retention time [19,20]. Polyesters such as poly(lactic-co-glycolic acid) (PLGA) are the materials generally used for nano-formulation. It is a novel mode of drug delivery system and considered to be nontoxic, biodegradable, biocompatible and non-immunogenic with a sustained drug-releasing ability in biological systems and has been approved by the U.S. Food and Drug administration for pharmaceutical application.

Abbreviations: CUR-NP, nanoparticle encapsulated curcumin; PLGA, poly(lactic-co-glycolic acid); NO, nitric oxide; eNPs, empty nanoparticles; DTH, delayed type of hypersensitivity reaction; KLH, Keyhole Limpet Hemocyanin; PBS, phosphate buffered saline; RBMI, Roswell Park Memorial Institute medium; Con-A, concanavalin-A; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); DMSO, dimethylsulphoxide; OD, optical density; SI, stimulation index; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent; PBST, Tween-20 phosphate buffer solution.

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Several studies showed that curcumin had ameliorative effects against arsenic-mediated toxicities. El-Demerdash et al. [21] and Yousef et al. [22] showed its effectiveness against arsenic-induced oxidative damage and systemic toxicity in rats. In a field trial in the arsenic-exposed population in West Bengal, it reduced oxidative stress and DNA damage [23]. It restored arsenic-mediated alterations in the levels of certain biogenic amines, their metabolites and nitric oxide (NO) in rats [24] and attenuated arsenic-induced apoptosis in murine splenocytes [25]. Further, its protective effect was demonstrated against cadmium-induced immunotoxicity in mice [26]. Recently, we reported its beneficial role in mitigating immunotoxic effects of cypermethrin in rats [27].

Given that curcumin as such has limited therapeutic efficacy because of the reasons mentioned above, we assumed that administering curcumin through a nano-delivery system would increase its effectiveness. To test this assumption, we evaluated whether the nanoformulation of curcumin could be more effective than free curcumin against arsenic-induced immune dysfunction in rats.

2. Materials and methods

2.1. Chemicals

Sodium meta-arsenite, curcumin, PLGA, and polyvinyl alcohol were purchased from Sigma-Aldrich (St. Louis, USA). All organic solvents were of HPLC grade. The other chemicals were of analytical or molecular grade.

2.2. Preparation of nanoparticle-encapsulated curcumin (CUR-NP)

Curcumin-loaded nanoparticles were prepared according to the solid-in-oil-in-water (s/o/w) emulsion technique [28,29] with minor modification. PLGA (45 mg) was dissolved in dichloromethane for 6 h to obtain a uniform PLGA solution. Normal curcumin was added to the PLGA solution and sonicated at 55 W for 1 min to produce the solid-in-oil primary emulsion. This emulsion was added to 20 ml of polyvinyl alcohol solution (1% w/v) and again sonicated at 55 W for 2 min to get the final solid-in-oil-in-water emulsion. The resulted nano-sized particles were stirred in the emulsion for 3 h for solvent evaporation. The final emulsion was centrifuged at 15,000 *g* for 15 min to remove the residual solvent. The nanoparticles obtained were washed thrice with deionized distilled water, and finally resuspended in deionized water and dried on a lyophilizer. The nanoparticles were stored at 4 °C till further use.

2.3. Particle size

The size of the nanoparticles was determined using a JOEL 1230 transmission electron microscope with a magnification of 80 kV [30] and the digital images were captured using a CCD camera (Danvers, MA). The lyophilized CUR-NP solution (1 mg/ml) was placed dropwise onto a copper grid with a filter paper. About 15 min after nanoparticle deposition, the grid was tapped with filter paper to remove excess water and stained using uranyl acetate (1%) for 10 min. The stained sample was air dried at room temperature for 2 h. The grid was then loaded into the transmission electron microscope and the size was determined.

2.4. Selection of dose and duration

Arsenic concentration was selected based on the maximum arsenic level (3.7 ppm) reported in groundwater in West Bengal, India [31]. Considering this as 4 ppm, its equivalent concentration of 25 ppm for rat was derived according to Reagan-Shaw et al. [32] and used for the study. Curcumin at 100 mg/kg bw was found to be effective in preventing biological dysfunctions induced by diverse environmental pollutants in rats [24,33]. We also reported the effectiveness of this dose in ameliorating cypermethrin-induced oxidative stress and immunotoxicity in rats [34,27]. We, therefore, used the dose of 100 mg/kg bw in the present study. Given the realities of population groups at risk in the arsenic-endemic areas, it is understood that the patients may need medical intervention at any time during the course of arsenic exposure. In an attempt to simulate such a situation, we gave arsenic exposure for a subchronic duration of 42 days, where curcumin co-administration was given during the last 14 days of arsenic exposure.

2.5. Animals and experimental design

Male Wistar rats (110–130 g) were obtained from the Animal Resource Section of the Institute. Rats were housed in polypropylene cages with chopped wheat straw as the bedding material. They were given standard rat chow (Amrut Feeds, Pranav Agro Industries, New Delhi, India) and water *ad libitum*. All the rats were kept in the laboratory conditions for a period of 7 days for acclimatization before the commencement of the experiment. The animals were handled and the study was conducted in accordance with the Institute guidelines for the protection of animal welfare.

Rats were randomly divided into five groups of six each. Group I was kept as the control and given only water. In Group II, rats were exposed to sodium arsenite (25 ppm) daily through drinking water for 42 days. Groups III, IV and V were treated with arsenic as in Group II, however, they were administered with empty nanoparticles (eNPs), curcumin (100 mg/kg bw) and CUR-NP (100 mg curcumin/kg bw in nanoparticle-encapsulated form), respectively, by oral gavage during the last 14 days, i.e., 29th to 42nd day of arsenic exposure. The rats were observed daily and body weights were recorded weekly. All the rats were killed under mild anesthesia at term for collection of blood and spleen.

2.6. Immunization and delayed type of hypersensitivity reaction (DTH)

Immunization was conducted as described by Exon et al. [35]. Briefly, 200 μl of 5 mg/ml Keyhole Limpet Hemocyanin (KLH) in sterile deionized water was injected subcutaneously into the base of the tail on the 29th and 36th days of arsenic exposure. For assessment of cell-mediated immunity, DTH reaction to KLH was carried out as per the method of Chen et al. [36]. The rats were injected 100 μl of 20 mg/ml heat-aggregated (80 °C for 1 h) KLH in phosphate-buffered saline (PBS) into the right hind footpad on the 42nd day. PBS (100 μl) was injected in to the left hind footpad that served as control. The footpad thickness was measured by a caliber 24 h after challenge with KLH and the difference in thickness between left and right footpads of the same animal was derived according to the formula $(R - L) / L \times 100$, where R is the thickness of the right footpad and L is the thickness of the left footpad. The response was expressed as percent increase in footpad thickness.

2.7. Separation of lymphocytes from spleen

Spleen was collected in sterile condition on the 43rd day. Splenocytes were harvested by repeated perfusion with sterile PBS using insulin syringe. The cell suspension was transferred into a 15 ml tube and centrifuged at 1200 rpm for 10 min. Cell pellets were then resuspended in PBS and layered over histopaque-1077 in the ratio of 1:1. The tubes were centrifuged at 1400 rpm for 40 min. The interface containing lymphocytes was collected in a fresh tube. The cells were washed twice with phenol red-free Roswell Park Memorial Institute (RPMI) medium (HEPES, 2 mM glutamine, 10% FBS and 1% antibiotic-antimycotic solution). The cell viability was counted by 0.1% trypan blue dye exclusion method.