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Unraveling the mechanism of neuroprotection of curcumin in arsenic induced cholinergic dysfunctions in rats

Pranay Srivastava [a], Rajesh S. Yadav [a,b], Lalit P. Chandravanshi [a], Rajendra K. Shukla [a], Yogesh K. Dhuriya [a], Lalit K.S. Chauhan [a], Hari N. Dwivedi [c], Aditiya B. Pant [a], Vinay K. Khanna [a,*]

[a] CSIR-Indian Institute of Toxicology Research, Post Box 80, MG Marg, Lucknow 226 001, India
[b] Department of Crimnology and Forensic Science, Harisingh Gour University, Sagar 470 003, India
[c] Babu Banarasi Das University, BBD City, Faizabad Road, Lucknow 227 015, India

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ABSTRACT

Earlier, we found that arsenic induced cholinergic deficits in rat brain could be protected by curcumin. In continuation to this, the present study is focused to unravel the molecular mechanisms associated with the protective efficacy of curcumin in arsenic induced cholinergic deficits. Exposure to arsenic (20 mg/kg body weight, p.o) for 28 days in rats resulted to decrease the expression of CHRM2 receptor gene associated with mitochondrial dysfunctions as evident by decrease in the mitochondrial membrane potential, activity of mitochondrial complexes and enhanced apoptosis both in the frontal cortex and hippocampus in comparison to controls. The ultrastructural images of arsenic exposed rats, assessed by transmission electron microscope, exhibited loss of myelin sheath and distorted cristae in the mitochondria both in the frontal cortex and hippocampus as compared to controls. Simultaneous treatment with arsenic (20 mg/kg body weight, p.o) and curcumin (100 mg/kg body weight, p.o) for 28 days in rats was found to protect arsenic induced changes in the mitochondrial membrane potential and activity of mitochondrial complexes both in frontal cortex and hippocampus. Alterations in the expression of pro- and anti-apoptotic proteins and ultrastructural damage in the frontal cortex and hippocampus following arsenic exposure were also protected in rats simultaneously treated with arsenic and curcumin. The data of the present study reveal that curcumin could protect arsenic induced cholinergic deficits by modulating the expression of pro- and anti-apoptotic proteins in the brain. More interestingly, arsenic induced functional and ultrastructural changes in the brain mitochondria were also protected by curcumin.

Introduction

Arsenic, a metalloid is ubiquitous in the environment due to its natural existence and extensive anthropogenic uses in the production of alloys, pesticides, pigments and antifouling paints (Mandal and Suzuki, 2002). Other potential uses of arsenic and arsenic products as leather preservative and in the manufacture of semiconductors, glass and ceramics and in the preparation of certain pharmaceuticals are well documented (Chitambar, 2010; Park et al., 2010). Although arsenic exists in three states (elemental — 0, trivalent — As^{III} and pentavalent — As^{V}), it is found in the environment both in the inorganic and organic forms (Rodríguez et al., 2003). Presence of high levels of arsenic in the ground water has affected significant population in many parts of the globe including India (Brammer and Ravenscroft, 2009; Brinkel et al., 2009; Mukherjee et al., 2006; Sultana et al., 2014). According to an estimate, it has been reported that over 140 million rural population from India, China, Myanmar, Pakistan, Nepal and Cambodia are prone to the risk of higher level arsenic exposure through groundwater (Brammer and Ravenscroft, 2009; Freeman, 2009). Due to associated health effects in humans following chronic arsenic exposure, the minimum permissible limit of arsenic in drinking water has been reduced to 10 ppb by the World Health Organization (Rodríguez-Lado et al., 2013). While arsenic has been classified as a human carcinogen resulting to skin lesions and cancers, arsenicosis is frequently reported in exposed individuals (Huang et al., 2013; Martinez et al., 2011). Interestingly, in a cross sectional study in Serbian population, Jovanovic et al. (2013) found an association between low level arsenic exposure through drinking water and development of type 2 diabetes. It has been found that arsenic exposure could affect internal body organs including the bladder, heart, liver, lung, and kidney while the brain is the soft target of the metalloid (El-Bahnasawy et al., 2013; Lech and Trela, 2005). Exposure to arsenic has been found to affect both the peripheral and central nervous systems (Frankel et al., 2009; Mathew et al., 2010). High level arsenic exposure may cause encephalopathy while chronic low level exposure may cause peripheral neuropathy (Sánchez-Peña et al., 2010). Further, incidences of neurobehavioral abnormalities including cognitive

* Corresponding author at: Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research, M.G. Marg, P.O. Box No. 80, Lucknow 226 001 U.P. India.
E-mail address: vkkhanna1@gmail.com (V.K. Khanna).

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deficits and deterioration in memory in arsenic exposed population are significantly enhanced (Rahman et al., 2007; Supapong and Sriratanabun, 2005; Wang et al., 2007).

A number of experimental studies have been carried out to understand the mechanisms associated with arsenic induced neurotoxicity (Kadeyala et al., 2013; Liu et al., 2013; Zhang et al., 2013). Of multiple mechanisms suggested, enhanced oxidative stress following arsenic exposure is one of the potential mechanism largely accepted (Jiang et al., 2013). Arsenic crosses the blood brain barrier and accumulates in the brain regions (Itoh et al., 1990; Tripathi et al., 1997). It has strong affinity with thiols and thus enhances oxidative stress by impairing the normal functioning of antioxidant enzymes (Yu et al., 2006). Stimulation of apoptotic factors involving signaling cascade leading to cell death through mitochondrial driven pathways associated with enhanced free radical generation has also been demonstrated (Andreyev et al., 2005; Flora et al., 2009; Haga et al., 2005; Kitchin and Ahmad, 2003; Mattson, 2007; Mishra and Palanivelu, 2008; Santra et al., 2007). Further, alterations in the process of synaptic transmission by modulating the levels of brain biogenic amines and sensitivity of neurotransmitter receptors following arsenic exposure has been reported (Liu et al., 2013; Zhang et al., 2013).

In view of increasing incidences of arsenic induced neurotoxicity and the consideration that a significant population is affected world over, there is a lot of concern if its neurotoxicity could be protected. While experimental studies involving pharmacological agents and herbal/natural extracts to assess their protective efficacy in arsenic induced neurotoxicity have been carried out extensively (Flora and Gupta, 2007; Gupta and Flora, 2006; Gupta et al., 2013; Shila et al., 2005b; Sinha et al., 2008), the molecular mechanisms involved in protective efficacy are not well understood. Curcumin, an active ingredient of *Curcuma longa* (turmeric) has a long history of its use as a spice/curry powder in cuisine in many Asian countries and in the traditional system of Indian medicine for the treatment of certain ailments (Krishnaswamy, 2008; Mito et al., 2011; Niu et al., 2013). Although potential of turmeric/curcumin due to wound healing and anti-inflammatory properties was recognized much earlier, its wide pharmacological spectrum including anti-ischemic, anti-aging, anti-mutagenic, anti-bacterial and anti-oxidant properties has made it more popular in recent years (Asher and Spelman, 2013; Guerra-Araiza et al., 2013). With the demonstration that curcumin may cross the blood brain barrier, a number of studies have been carried out to assess the protective potential of curcumin in psychiatric, neurological and neurotoxicological disorders (Aggarwal and Sung, 2009; Maheshwari et al., 2006; Shukla et al., 2008; Strimpakos and Sharma, 2008; Thapa et al., 2013; Wang et al., 2005). *In vitro* and *in vivo* studies have shown that curcumin has the potential to affect cellular processes by modulating the biochemical pathways (Chin et al., 2013; Hasan et al., 2014; Pyun et al., 2013). Interestingly, free radical scavenging activity of curcumin has been attributed strongly with these biochemical mechanisms (Kuo et al., 2011; Lee et al., 2013; Rajasekar et al., 2013). Cytoprotective efficacy of curcumin in MPP^+ induced neurotoxicity *via* activation and modulation of anti-apoptotic and Bcl-2-mitochondria-ROS iNOS pathway has been observed *in vitro* (Chen et al., 2006). In addition, involvement of various transcription factors like Nrf2 and AP-1 in the neuroprotective efficacy of curcumin have also been demonstrated (Shishodia et al., 2005). While our previous studies demonstrated that arsenic induced brain cholinergic and dopaminergic deficits are associated with enhanced oxidative stress and could be protected by curcumin (Yadav et al., 2009, 2010, 2011), not much is known about the molecular mechanisms involved. Arsenic targets the mitochondria and enhances the apoptosis by affecting the apoptotic factors and stress markers. However, the mechanism of protective potential of curcumin in arsenic induced changes is not understood. Also, it is not clear if arsenic induced ultrastructural damage in brain could be protected by curcumin. The present study has therefore been aimed to investigate and decipher the molecular mechanisms involved in the neuroprotective efficacy of curcumin in arsenic induced neurotoxicity in rats.

Materials and methods

Animals and treatment

Male rats of Wistar strain weighing 180 ± 20 g obtained from the animal breeding colony of CSIR — Indian Institute of Toxicology Research (IITR), Lucknow were used in the present study. The experimental rats were housed in an air conditioned room at 25 ± 2 °C with a 12-hour light/dark cycle under standard hygiene conditions and fed pellet diet obtained commercially and water *ad libitum*. To assess the neuroprotective efficacy of curcumin in arsenic induced neurotoxicity, rats were treated with arsenic or curcumin alone or in combination as per following schedule:

Group I — Rats treated with arsenic as sodium arsenite (dissolved in distilled water, 20 mg/kg body weight, p.o., daily for 28 days)

Group II — Rats treated with curcumin (suspended in 2% gum acacia, 100 mg/kg body weight, p.o., daily for 28 days)

Group III — Rats treated with arsenic and curcumin in combination identically as in Groups I and II

Group IV — Rats treated with vehicle (2% gum acacia) for the duration of the treatment to serve as controls

The protocol for the study was approved by the Institutional Animal Ethics Committee of CSIR-IITR, Lucknow and all experiments have been carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India. For neurochemical studies, rats were sacrificed by decapitation 24 h after the last dose of treatment. The brain in each case was removed quickly, washed in ice-cold saline and dissected into frontal cortex and hippocampus following the standard procedure (Glowinski and Iversen, 1966). For ultrastructural studies, perfused brains from different treatment groups were used.

Neurochemical studies

Expression of cholinergic–muscarinic-2 (CHRM2) receptor gene in frontal cortex and hippocampus. RNA extraction and cDNA synthesis. Total RNA from the frontal cortex and hippocampus of rats exposed to arsenic, curcumin and their simultaneous treatment was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA). The integrity of isolated RNA was checked by electrophoresis on 2% denatured agarose gel. The concentration of RNA was measured using NanoDrop ND-1000 (Thermo Scientific, Wilmington, DE, USA). RNA (1 μg) was reverse transcribed into cDNA using High Capacity Reverse Transcriptase kit (Applied Biosystems, USA) following the protocol provided by the manufacturer.

Quantitative Real Time PCR (qRT-PCR) analysis. Expression of CHRM2 receptor gene was analyzed by qRT-PCR (ABI 7900HT, Applied Biosystems, USA) using SYBR green dye (Applied Biosystems, USA) running 40 cycles using following protocol: one cycle of denaturation (95 °C for 10 s) of 40 cycles, annealing (60 °C, 10 s) and extension (72 °C, 45 s). A final extension (72 °C, 5 min) was performed. The sequences of primers used were — CHRM2, FP 5-GGCAAGCAAGAGTAGA ATAAA-3 and CHRM2, RP 5-GCCAACAGGATAGCCAAGATT-3 and GAPDH, FP 5-CGGGAAGCTTGTGATCAATGG-3 and GAPDH, RP 5-GGCA GTGATGCCATGGACT G-3. (Borges et al., 2001). Exposure induced alteration in mRNA expression of CHRM2 was given as Relative Quantification in comparison to control.

Assessment of reactive oxygen species generation in rat frontal cortex and hippocampus. Amount of reactive oxygen species generation was assessed fluorometrically by measuring the conversion of DCFH-DA dye into highly fluorescent DCF product by cellular peroxides (including hydrogen peroxide). The assay was performed following the method of