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Attenuation of arsenic neurotoxicity by curcumin in rats

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ABSTRACT

In view of continued exposure to arsenic and associated human health risk including neurotoxicity, neuroprotective efficacy of curcumin, a polyphenolic antioxidant, has been investigated in rats. A significant decrease in locomotor activity, grip strength (26%) and rota-rod performance (82%) was observed in rats treated with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) as compared to controls. The arsenic treated rats also exhibited a decrease in the binding of striatal dopamine receptors (32%) and tyrosine hydroxylase (TH) immunoreactivity (19%) in striatum. Increased arsenic levels in corpus striatum (6.5 fold), frontal cortex (6.3 fold) and hippocampus (7.0 fold) associated with enhanced oxidative stress in these brain regions, as evident by an increase in lipid perioxidation, protein carbonyl and a decrease in the levels of glutathione and activity of superoxide dismutase, catalase and glutathione peroxidase with differential effects were observed in arsenic treated rats compared to controls. Simultaneous treatment with arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) and curcumin (100 mg/kg body weight, p.o., 28 days) caused an increase in locomotor activity and grip strength and improved the rota-rod performance in comparison to arsenic treated rats. Binding of striatal dopamine receptors and TH expression increased while arsenic levels and oxidative stress decreased in these brain regions in co-treated rats as compared to those treated with arsenic alone. No significant effect on any of these parameters was observed in rats treated with curcumin (100 mg/kg body weight, p.o., 28 days) alone compared to controls. A significant protection in behavioral, neurochemical and immunohistochemical parameters in rats simultaneously treated with arsenic and curcumin suggest the neuroprotective efficacy of curcumin.

Introduction

Extensive applications of arsenic in mining, smelting and refining of certain ores have distributed it into the environment (Goyer, 1991; Polissar et al., 1990; Klaassen, 2001). Burning of coal has also contributed to disperse arsenic in the environment. High levels of arsenic in ground water have been detected in some regions in India and several other countries and thus pose health risk to humans (Das et al., 1995; NRC, 2001; Hassan et al., 2003). Exposure to arsenic in humans has also been reported through folk medicines and by consuming contaminated food material particularly sea food (ATSDR, 2005; WHO, 1992; Francesconi and Edmonds, 1987; Foa et al., 1984; Vahidnia et al., 2007). Although both organic and inorganic forms of arsenic exist in nature, humans are mainly exposed from inorganic arsenic through drinking water and occupational sources. Arsenic and its inorganic compounds have long been known to be neurotoxic (Vahidnia et al., 2007). Peripheral neuropathy following arsenic exposure is well documented (Chhuttani and Chopra, 1979; Schoolmeester and White, 1980; Brouwer et al., 1992; Heaven et al., 1994). A decrease in peripheral nerve conduction velocity has been reported following chronic exposure to arsenic dust (Blom et al., 1985; Vahidnia et al., 2007). An association between arsenic ingestion and increased risk of microvascular diseases including neurological disorders has been reported (Chiou et al., 2005). Gharibzadeh and Hoseini (2008) suggested that arsenic exposure may be a risk factor for Alzheimer's disease by inducing apoptosis in cortical neurons.

Arsenic easily crosses the blood brain barrier (Tripathi et al., 1997) and accumulates in the brain leading to neurobehavioral abnormalities (Itoh et al., 1990). Although not much information about the precise target of arsenic in brain is known, basal ganglia has been shown to be quite vulnerable (Ghafgazi et al., 1980; Rodriguez et al., 2001). Studies have been carried out in whole brain (Flora et al., 2005; Gupta and Flora, 2006) and brain regions to understand the mechanism of arsenic induced neurotoxicity (Shila et al., 2005a, 2005b, 2005c). It was observed that arsenic has marked effect on corpus striatum, cortex and hippocampus (Shila et al., 2005c). Delayed maturation of Purkinje cells and their defective migration have been reported in rats exposed to sodium arsenite during rapid

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brain growth period from postnatal days 4 to 10 (Dhar et al., 2007). Impaired learning and memory in arsenic exposed individuals and children have been reported (Danan et al., 1984; Calderon et al., 2001). Alteration in motor behavior has also been reported in arsenic exposed rats (Rodriguez et al., 2001, 2003).

A number of studies have been carried out to understand the biochemical mechanisms involved in arsenic induced neurotoxicity. Levels of dopamine, norepinephrine and serotonin have been found to be altered following arsenic exposure in experimental studies suggesting the role of biogenic amines in the neurotoxicity of arsenic (Tripathi et al., 1997; Kannan et al., 2001). Besides effect on the catacholaminergic system, enhanced oxidative stress associated with decreased antioxidant defense in the brain has been reported in arsenic neurotoxicity (Gupta et al., 2005; Shila et al., 2005a, 2005b; Flora and Gupta, 2007; Sinha et al., 2008). Arsenic enhances generation of free radicals leading to increased lipid peroxidation, protein carbonyls and decreased activity of superoxide dismutase and other enzymes involved in antioxidant defense in rat brain. Besides, arsenic has high affinity to GSH and thus enhances vulnerability towards oxidative stress by causing an imbalance between pro-oxidant and antioxidant homeostasis (Aposhian and Aposhian, 1989; Wang et al., 1996; Chen et al., 1998; Shila et al., 2005a, 2005b, 2005c). Chronic exposure to arsenic in rats was found to decrease the production of brain nitric oxide associated with an increase in the production of reactive oxygen species (Zarazua et al., 2006). In view of the continued exposure to arsenic in humans, there is a lot of interest in investigating if its neurotoxicity could be prevented.

Plant extracts and pharmacological agents have been used to investigate their neuroprotective efficacy in arsenic induced neurotoxicity with an aim to asses their antioxidant potential but with variable results (Gupta et al., 2005; Gupta and Flora, 2006; Flora and Gupta, 2007; Shila et al., 2005a; Sinha et al., 2008). Turmeric is extensively used as a spice, food preservative and coloring material in different parts of the world especially in Asian countries. It has been used as an additive by food industries in U.K. (Strimpakos and Sharma, 2008). Curcumin, present in turmeric, is an active ingredient and known to possess multiple pharmacological properties such as anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-ischemic, hypotensive and antioxidant (Arora et al., 1971; Kim et al., 1998; Polasa et al., 2004; Dikshit et al., 1995; Jones and Shoskes, 2000; Lee et al., 2005; Al-Omar et al., 2006; Aggarwal et al., 2006; Maheshwari et al., 2006; Shukla et al., 2007; Goel et al., 2008; Hatcher et al., 2008; Strimpakos and Sharma, 2008). Curcumin has been found to be effective in the treatment of Alzheimer's dementia, neuroleptic-induced tardive dyskinesia and chemical induced neurotoxicity including lead and cadmium (Garcia-Alloza et al., 2007; Bishnoi et al., 2008; Dairam et al., 2007; Shukla et al., 2003; Daniel et al., 2004). Due to high safety of curcumin in phase I trials on human volunteers, clinical trials are being done to assess its therapeutic potential in disease state (Chainani-Wu, 2003; Aggrawal and Sung, 2009). Recently, Yousef et al. (2008) observed that arsenic induced biochemical alterations in the brain and liver of rats could be protected by curcumin. Since enhanced oxidative stress has been reported to be one of the important mechanisms in arsenic neurotoxicity, the present study with curcumin has been carried out to investigate its neuroprotective efficacy because of its antioxidant potential focusing on the parameters related to oxidative stress. To further understand the potential of arsenic on dopaminergic alterations and neuroprotective efficacy of curcumin, if any, effect on dopamine receptors and related behaviors was studied. In view of the vulnerability of basal ganglia and associated risk of Alzheimer's disease to arsenic, studies were carried out on corpus striatum, a brain area controlling dopaminergic mechanisms and frontal cortex and hippocampus, functionally involved in Alzheimer's disease in the present investigation.

Materials and methods

Animals and treatment

Female rats (180 ± 20 g) of Wistar strain, obtained from the animal breeding colony of Indian Institute of Toxicology Research (IITR), Lucknow were used for the study. Rats were housed in an air conditioned room at a temperature 25 ± 2 °C with a 12-hour light/dark cycle under standard hygiene conditions and had free access to pellet diet (Ashirwad Industries, Chandigarh, India) and water. The animals were randomly divided into four groups. Rats in Group I were treated with arsenic as sodium arsenite (dissolved in distilled water, 20 mg/kg body weight, p.o., daily for 28 days). In Group II, rats were treated with curcumin 99% pure procured from Kancor, Kerala, India (suspended in 2% gum acacia, 100 mg/kg body weight, p.o., daily for 28 days). Rats in Group III were simultaneously treated with arsenic and curcumin in combination identically as in Groups I and II. In Group IV, rats were treated with 2% gum acacia dissolved in distilled water p.o. for the duration of the treatment to serve as controls.

Behavioral studies were carried out as per plan after the last dose of treatment. A set of five rats randomly selected from each treatment group was used to assess spontaneous locomotor activity 24 h after the last dose of treatment. The same set of rats was used to measure grip strength, 1 h after the spontaneous locomotor activity test. Rota-rod test was carried out in a separate set of five rats randomly selected from each treatment group. For neurochemical studies, rats were terminated by cervical decapitation around 24 h after the last dose of treatment. Brains were taken out quickly, washed in ice cold saline and dissected into regions (corpus striatum, frontal cortex and hippocampus) following the standard procedure (Glowinski and Iversen, 1966). Brain regions were processed immediately for the assay of parameters related with oxidative stress. For the assay of dopamine (DA) — D_2 receptors, corpus striatum was kept frozen at −20 °C and processed within 72 h. For TH immunohistochemistry, brains from the perfused rats from each treatment group were processed within 4–5 days following the standard protocol. Arsenic levels in the brain regions of treated rats were estimated within 6 days after the termination of treated animals. The study was approved by the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Toxicology Research, Lucknow and all experiments were 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.

Behavioral studies.

(i) **Spontaneous locomotor activity:** Spontaneous locomotor activity in rats was carried out using computerized Actimot (TSE, Germany) following the method as described by Ali et al. (1990). Effect on different parameters including total distance travelled, resting time, stereotypic time, time moving and rearing was studied in rats in the control and treated groups.

(ii) **Rota-rod performance:** Effect of arsenic and the protective effect of curcumin on motor in coordination were studied in rats using Rotomex (Columbus Instruments, USA) and the time of fall from the rotating rod was monitored following the standard procedure (Rogers et al., 1997).

(iii) **Grip strength:** A computerized grip strength meter (TSE, Germany) was used to measure the forelimb grip strength in the control and treated rats following the standard procedure as described by Terry et al. (2003).

Neurochemical studies. Assay of dopamine receptors and other parameters related to oxidative stress was carried out following the standard protocol to understand the protective efficacy of curcumin in arsenic induced neurotoxicity.