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Original Article

Dendritic processes as targets for arsenic induced neurotoxicity: Protective role of curcumin

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ABSTRACT

Introduction: Microtubule associated protein2 (MAP2) plays a vital role in morphological stabilization and plasticity of the neuronal dendritic processes. Any alteration in the expression of this protein following exposure to environmental contaminants such as arsenic (*iAs*) could induce functional deficits in neurons. In India, over 1.5 million people are exposed to *iAs* with over 200,000 reported cases of arsenicosis. Oxidative stress has been identified as one of the key factors underlying *iAs* induced toxicity. Hence, the need of the hour is to identify cost effective and safe therapeutic approaches for combating *iAs* induced adverse effects. The present study aimed at determining the ameliorative potential of Curcumin (Cur) supplementation on dendritic profile of cerebellar Purkinje cells in rats subjected to *iAs* exposure during postnatal period.

Methods: Mother reared rats were divided into control and experimental groups (receiving $NaAsO_2$ alone or along with Cur by intraperitoneal route from postnatal day (PND) 1–21. Cerebellar tissue obtained from perfusion fixed animals was processed for Cresyl Violet staining and immunohistochemical localization of MAP2.

Results: An overall decrease in molecular layer thickness (MLT) of cerebellar cortex along with disrupted morphology of Purkinje dendritic processes was evident in *iAs* alone treated animals as compared to controls and Cur co-treated animals. Also, decrease in MAP2 immunostained area (%) was noted in the ML of *iAs* alone treated animals.

Discussion: Preliminary observations suggest modulating effect of Cur on MAP2 expression and dendritic morphology of cerebellar Purkinje cells in rats following $NaAsO_2$ exposure.

1. Introduction

Exposure to various environmental contaminants is emerging as a matter of great concern all over the globe. Millions of people across various continents get exposed to *iAs* by consuming ground water contaminated with high levels of *iAs*.[1] There have been reports of adverse effects on various organ systems in humans and animal models following exposure to *iAs*.[2] Besides contaminated water, consumption of grains (especially rice) grown in areas where *iAs* contaminated water is used for irrigation, constitutes additional medium of exposure to *iAs*.[3,4] In India, over 1.5 million people are exposed to high levels of *iAs* with over 200,000 reported cases of arsenicosis. It is worthwhile to mention here that pregnant women comprise a significant segment of this population, especially in the endemic areas.[1]

Developmental exposure to environmental contaminants during critical periods of Central Nervous System (CNS) development is seen as an open challenge. A number of cellular processes ranging from neurite formation (neuritogenesis) to neuro-morphogenesis[5] are highly susceptible to various exogenous as well as endogenous insults. Amongst various cell organelles, stability of cellular skeleton is considered critical for healthy maintenance of cellular architecture, intracellular movements, cell division etc.[6,7] Being extensively localized in the dendrites of the nerve cells, Microtubule Associated Protein 2 (MAP2), plays a key role in neural transmission by facilitating the assembly of microtubules.[8] MAP2 contributes actively in morphological stabilization and plasticity of the dendritic processes[9] hence, considered as one of the important markers for structural integrity. The observations of our earlier study suggested decreased cognitive and exploratory ability in rat pups exposed to sodium arsenite ($NaAsO_2$) during postnatal

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period.[10] For understanding the structural basis underlying *iAs* induced functional deficits, we studied the effect of postnatal *iAs* exposure on expression pattern of MAP2 in cerebellar cortex of rat pups. Since, oxidative stress has been reported as one of the factors underlying *iAs* induced toxicity, the aim of the present study was to determine the effects of curcumin (Cur) (bioactive component of turmeric) supplementation on *iAs* induced effects on MAP2 expression (marker of neuronal structural integrity) in cerebellar cortex of these animals.

2. Material and methods

Ethical clearance from the Institute Animal Ethical Committee (IAEC 594/11) was obtained for procuring pregnant Wistar rats (gestation day 18–19). The animals were housed in temperature (20 °C-25 °C) and humidity (50-60%) controlled rooms within Central Animal Facility with 12 *h* light/dark cycle and fed on standard rodent diet with *ad libitum* access to drinking water. The guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) were strictly followed for the care of the animals. The delivery status of the animals was checked daily at 10 A M and 4 PM and the day of delivery of pups was designated as postnatal day (PND) 0. The mother reared pups were grouped as normal controls (Ia- with no treatment) and sham controls [Ib- sterile water; Ic- dimethyl sulfoxide (DMSO Sigma Aldrich D5879)]. Animals belonging to the experimental groups received $NaAsO_2$ alone [IIa- 1.5; IIb- 2.5 mg/kg body weight (bw)] (Sigma Aldrich 71,287); whereas animals in groups IIIa and IIIb received Cur (150 mg/kg bw) (Sigma Aldrich C1386) along with 1.5 and 2.5 mg/kg bw $NaAsO_2$ (Fig. 1). The test substances ($NaAsO_2$ and Cur) were administered once daily by intraperitoneal (i.p.) route from PND 1–21 with half an hour interval in between.[11] i.p. route was considered for ensuring the requisite dose delivery of the test substance and to avoid variation in exposure doses. To the best of our knowledge, there are no reports suggestive of Cur induced toxicity at the dosage used in the study. During the entire experimental period, the animals were observed for signs of normal developmental features. The animals were sacrificed on PND 22 by perfusion fixation (0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer) and the cerebellar tissue obtained was processed for paraffin and cryo sectioning (n = 12/group).

2.1. Morphology and morphometry

The fixed cerebellum was dehydrated in ascending grades of ethanol [70% (overnight), 80% [30 min], 90% (30 min), 96% (15 min) and 96% (15 min)]. Tissue was cleared in xylene (30 min) and embedded in paraffin.7 μm thick serial sections were cut (Rotary microtome- Shandon AS325) and processed for CV staining. Briefly, the sections were dewaxed in xylene (5 min) and hydrated in decreasing concentrations of ethanol (96%, 90%, 80% and 70%) (5 min each). The slides were immersed in CV stain (Sigma Chemicals) (5 min) and then dehydrated by passing through increasing grades of ethanol 30%, 50%, 70%, 90%, 96% (3 min each). The slides were differentiated in acid alcohol, cleared in xylene and mounted in DPX. The stained sections (vermal and paravermal regions) were observed under bright field Nikon E-600 microscope fitted with Nikon Digital Camera System (DS-Fil-U2) for morphological features. Morphometric parameters were determined by the attached image analysis system (Nikon Imaging Software, NIS-Elements-AR 3.10). In the cerebellar cortex, Molecular Layer thickness (MLT) was measured (μm) as a perpendicular line drawn from the outer edge of ML to outer edge of Purkinje cell layer (PCL). A total of 10 reference areas/ section were randomly selected and 5 sections per animal were analysed (n = 6/ group). The first section was randomly chosen and the subsequent sections were every 20th from that.[12]

2.2. Immunohistochemistry and semi quantitative analysis

Fixed cerebellar tissue was subjected to cryoprotection in graded sucrose solution (15% and 30%), at 4 °C. Cryocut (HS 525, Microm GmbH, Germany) sagittal sections (30 μm) were collected in 0.1 M PB and processed by free floating immunohistochemical technique following the standardized protocol.[10] Antigen retrieval was done using 0.5% Sodium Dodecyl Sulphate (SDS) and quenching of endogenous peroxidase was carried out in 0.3% hydrogen peroxide. After washing (0.1 M PBS), blocking was done in normal goat serum (Jackson Laboratories, USA) (1 *h*, at RT) followed by overnight incubation in primary antibody (mouse monoclonal anti-microtubule associated protein 2 MAP2, Santa Cruz, USA 56561, 1:200). Ultravision Plus Detection system kit (Thermo Scientific TP-060-HLX) and DAB kit (Bio SB. BSB 0017) were used for visualization of immune complexes. The sections were dehydrated and mounted on gelatin coated slides.

The mid-sagittal sections (vermal and para-vermal regions) presenting well defined lobules were considered for semi-quantitive analysis of IHC expression. The mean cumulative grey values were calculated on a grey scale of 0–255 where (0) represented black and 255 represented white.[13,14] A rectangular grid (200 μm x 50 μm) was superimposed on randomly selected reference areas in ML and the area surrounding the grid was cropped off. The percentage of MAP2 immuno-stained area (reflecting mainly the dendritic arborization of the PCs in the ML) was determined in the region of interest (ROI) and expressed

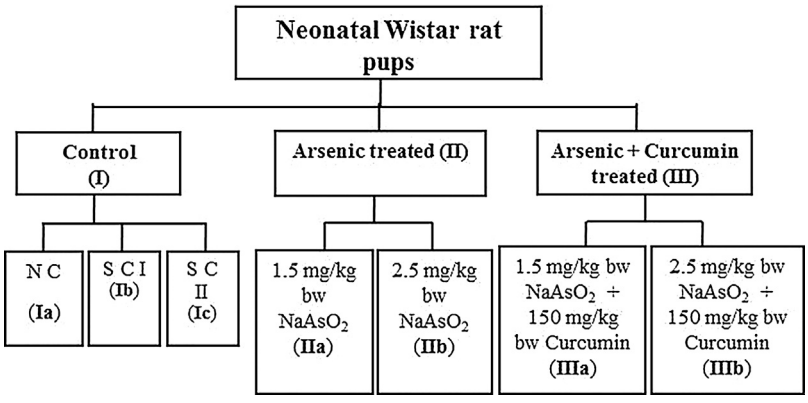

Fig. 1. Showing the grouping of mother reared rat pups.