Food and Chemical Toxicology 82 (2015) 64–71

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Sodium tungstate induced neurological alterations in rat brain regions and their response to antioxidants

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ARTICLE INFO

Article history:
Received 18 February 2015
Accepted 7 May 2015
Available online 14 May 2015

Keywords:
Sodium tungstate toxicity
Oxidative stress
Antioxidants
Flavonoids
Brain regions
Neurotoxicity

ABSTRACT

Tungsten, recognized recently as an environmental contaminant, is being used in arms and ammunitions as substitute to depleted uranium. We studied the effects of sodium tungstate on oxidative stress, few selected neurological variables like acetylcholinesterase, biogenic amines in rat brain regions (cerebral cortex, hippocampus and cerebellum) and their prevention following co-administration of N-acetylcysteine (NAC), naringenin and quercetin. Animals were sub-chronically exposed to sodium tungstate (100 ppm in drinking water) and orally co-supplemented with different antioxidants (0.30 mM) for three months. Sodium tungstate significantly decreased the activity of acetylcholinesterase, dopamine, nor-epinephrine and 5-hydroxytryptamine levels while it increased monoamine oxidase activity in different brain regions. Tungstate exposure produced a significant increase in biochemical variables indicative of oxidative stress while, neurological alterations were more pronounced in the cerebral cortex compared to other regions. Co-administration of NAC and flavonoids with sodium tungstate significantly restored glutathione, prevented changes in the brain biogenic amines, reactive oxygen species (ROS) and TBARS levels in the different brain regions. The protection was more prominent in the animals co-administered with NAC. We can thus conclude that sodium tungstate induced brain oxidative stress and the alterations in some neurological variables can effectively be reduced by co-supplementation of NAC.

1. Introduction

Tungsten (W), one of the rare metals, occurs naturally as tungstate and is the most common form in ecological and biological systems. Tungsten can be released naturally in the environment as a result of weathering of rocks and soils or from sewage sludge, fertilizers, municipal solid waste ash, industrial wastes that contain tungsten. The recent increase in use of Tungsten due to its broad applications (viz. filaments of light bulb and X-ray tubes, electrodes in TIG welding, industrial catalysts, in radiation shielding, in arms and ammunitions as substitute to lead and depleted uranium etc.) and its simultaneous release has created a scientific concern regarding its impact on the environment (Johnson et al., 2010). Thus, primarily people working in these industries have high risk of exposure, followed by population living near industries, then those who get exposed to tungsten in food and drinking water (ATSDR, 2005). Exposure to the contents of arms and ammunitions used in war remains as another means of exposure for military personnel and to the exposed civilians in that area.

Long term health effects may occur when tungsten compounds enter frequently (in small quantity) into the system. Neurological and respiratory disorders were reported among workers in a metal industry, miners and also in French soldiers exposed to tungsten (Centeno et al., 2014; Chen et al., 2005; Jordan et al., 1990). There is thus an increased human health concern due to the use of tungsten. Tungsten was detected in ground water, tap water as well as in urine of children and adults suffering from childhood leukemia clusters (Marquet et al., 1996; CDC, 2003; Pritsos et al., 2009; Rubin et al., 2007; Seiler et al., 2005). Rubin et al. (2007) reported high incidences of childhood leukemia in Fallon, Nevada, and other western U.S. towns because of tungsten exposure via drinking water. It has been associated with the onset of acute lymphocytic leukemia clusters (Pritsos et al., 2009; Sheppard et al., 2007; Walker and Fosbury, 2009). It has been reported that tungsten gets accumulated in the kidneys, liver, ovaries, uterus, prostate, pancreas, lung, heart, fat and muscle, with the highest concentrations detected in the spleen and bone, following single oral dose (ATSDR, 2005; Lagarde and Leroy, 2002). Tungsten exposure is trailed with various vascular anomalies and morphological changes in rat brain and the same were correlated with neurobehavioral perturbations (Agarwal et al., 2011). Furthermore tungsten exposed subjects were also reported with neurobehavioral deficits like altered locomotor activity (McInturf et al., 2008) and declined memory (Chen et al., 2005). Neurotransmitters play a major role in the regulation and maintenance of behavior and cognition via

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http://dx.doi.org/10.1016/j.fct.2015.05.003
0278-6915/

multiple feedback mechanisms regulating phasic and tonic components of neurotransmission, synaptic regulation and integration recruited by the specific behavioral and cognitive activity (Sarter et al., 2007). Dysregulation in the normal level of neurotransmitters results in various behavioral, cognitive and neuropsychiatric disorders (Sarter et al., 2007). However, literature supporting the alteration of neurotransmitters in brain remains unexplored.

In our previous studies we (Sachdeva and Flora, 2014; Sachdeva et al., 2013) have reported oxidative stress as one of the major mechanism involved in the toxic manifestations of sodium tungstate in blood, liver and spleen. Long term health effects may occur when tungsten compounds enter frequently in the system or might be present in the form of embedded shrapnel leading to continuous production of reactive oxygen species. In a variety of neurodegenerative/neurotoxic conditions (Reiter et al., 2001), the role of free radicals in altering neurotransmitter level in the brain has already been explained. Antioxidant supplementation leads to cascade of reactions that are essential for the neutralization of reactive radicals which in turn reduces oxidative stress. Thus, administration of antioxidant can validate our findings and can be further used as benchmark for establishing preventive and therapeutic aspects against tungsten induced neurotoxicity.

Few reports of sodium tungstate toxicity through intravenous and intraperitoneal routes are available in the literature (Ando et al., 1989). Risk to developing brain has also been demonstrated as tungsten readily crosses the placenta into the fetuses of pregnant rats and is present in the milk of exposed dams (Lamer et al., 2000). However, information regarding its toxic effect in altering neurotransmitters following oral route in adult rats is lacking. Thus, in the present study we investigated the effects of oral exposure to sodium tungstate on biochemical variables indicative of oxidative stress, alterations in the levels of neurotransmitters and acetylcholinesterase activity in adult rat brain regions. Further, we also evaluated free radicals scavenging activity of different antioxidants viz. quercetin, N-acetylcysteine and naringenin in preventing appearance of above mentioned alterations during concomitant administration.

2. Materials and methods

2.1. Chemicals

Sodium tungstate hydrated ($Na_3WO_4.2H_2O$; Product no. 20684; lot # 2178 6802 -1, purity – 96%) was procured from Fischer Scientific (Qualigens Fine Chemicals, Mumbai, India). All other laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or BDH Chemicals (Mumbai, India). Triple distilled water prepared by Millipore (New Delhi, India) was used throughout the experiment to avoid contamination, and for the preparation of reagents and buffers used for various biochemical assays in the study.

2.2. Animals and treatment

Male Wistar rats, weighing approximately 100–120 g were used in the study. Animals were obtained from the animal house facility of the Defence Research and Development Establishment (DRDE), Gwalior. The Animal Ethical Committee of DRDE, Gwalior, India also approved the protocols for the experiments. Prior to the dosing, they were acclimatized for 7 days. All animals received humane care in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Briefly, animals were kept in polypropylene cages with clean dust free rice husk. The animals were housed in stainless steel cages in an air-conditioned room with temperature maintained at 25 ± 2 °C and relative humidity 60 ± 10%. Animals' rooms were illuminated with 300 lux light, alternating with 12 h darkness light from 06:00 to 18:00 h and vice versa. Rats were allowed standard chow diet (Ashirwaad Industries, Ropar, Punjab, India) throughout the experiment and water *ad libitum*.

2.3. Treatment schedule

Rats were divided into eight groups of 10 rats each and were treated as below for 3 months (five days a week):

Group I: Normal animal, received normal water
Group II: Sodium tungstate, 100 ppm in drinking water, daily
Group III: Quercetin alone, orally, once, daily (0.30 mM)
Group IV: N-acetylcysteine (NAC) alone, orally, once, daily (0.30 mM)
Group V: Flavonoid (naringenin) alone, orally, once, daily (0.30 mM)
Group VI: Sodium tungstate + Quercetin as in group II and group III, respectively
Group VII: Sodium tungstate + NAC (as in group II + group IV, respectively)
Group VIII: Sodium tungstate + Flavonoid (naringenin) (as in group II + group V, respectively)

The doses for sodium tungstate and equimolar doses of antioxidants quercetin, n-acetylcysteine and Naringenin were selected based on earlier publication (Sachdeva and Flora, 2014). After three months of treatment all animals were sacrificed under light ether anesthesia. Five rats were used for studying changes in acetylcholinesterase and biogenic amine levels in brain regions while remaining five rats were used for studying other biochemical variables. Blood was collected by cardiac puncture in heparinized vials. Brain were rinsed in cold saline, blotted, weighted and used for various biochemical variables.

2.4. Dissection of brain parts

Brains were quickly dissected out, placed on a glass plate resting over crushed ice for the separation of various regions (cortex, cerebellum and hippocampus) (Scheuhammer and Cherian, 1982). The brain parts were homogenized in 19 volumes of 0.1 M phosphate buffer (pH 7.4). Rat brain homogenates were first centrifuged at 1500 × *g* for 10 min to discard nuclei and cell debris and then at 14,000 × *g* for 10 min in cold conditions. The supernatant fractions obtained were used for various parameters described below, in three different brain regions.

2.5. Reactive Oxygen Species (ROS)

Amount of ROS in blood was measured using 2′, 7′-dichlrofluorescein diacetate (DCF-DA) that gets converted into highly fluorescent DCF by cellular peroxides (including hydrogen peroxide). The assay was performed as described by Socci et al. (1999). For estimation of ROS in blood, 5% RBC hemolysate was prepared and diluted to 1.5% with ice-cold 40 mM tris–HCl buffer (pH 7.4). Similarly for the estimation of ROS in tissues, 10% tissue homogenate was prepared. The tissue was homogenized (10 mg) in 1 ml of ice-cold 40 mM tris–HCl buffer (pH 7.4), further diluted to 0.25% with the same buffer and placed on ice. Then after, 40 µl of 1.25 mM DCF-DA in methanol were added for ROS estimation. All samples were incubated for 15 min in a 37 °C water bath. The diacetate form, H_2DCFDA and its acetomethyl ester H_2DCFDA-AM are taken up by cells where non specific cellular esterases act upon it to cleave off the lipophilic groups, resulting in a charged compound believed to be trapped inside the cell. Oxidation of H2DCF by ROS converts the molecule to 2′, 7′ dichlorodihydrofluorescein (DCF), which is highly fluorescent. Fluorescence was determined at 488 nm excitation and 525 nm emission using a fluorescence plate reader (Tecan Spectra Fluor Plus).

2.6. Tissue Thiobarbituric acid reactive substances (TBARS)

Measurement of lipid peroxidation was done by the method described by Ohkawa et al. (1979). One milliliter of tissue homogenate, prepared in 0.15 M KCl, was incubated for 1 h at 37 °C followed by addition of 10% TCA, mixed thoroughly and centrifuged at 3000 rpm for 10 min. 1 ml TBA was added to 1 ml supernatant and the tube was kept in boiling water bath for 10 min till the pink color appeared. One milliliter of double distilled water was added to after cooling the tubes and light absorbance was measured at 532 nm.

2.7. Tissue reduced glutathione (GSH) and oxidized glutathione (GSSG)

Tissue GSH and GSSG levels were measured as described by Hissin and Hilf (1974). Briefly, 0.25 g of tissue sample was homogenized on ice with 3.75 ml of phosphate–EDTA buffer and 1 ml of 25% HPO_3 which was used as a protein precipitant. The total homogenate was of H_2O_2 (1 mM) and 0.3 ml of tissue supernatant. After incubation at 37 °C for 15 min, reaction was terminated by addition of 0.5 ml of 5% TCA. Tubes were centrifuged at 1500 × *g* for 5 min and supernatant was collected. 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of the reaction supernatant. After mixing well, light absorbance was recorded at 420 nm.

2.8. Tissue glutathione peroxidase (GPx)

Glutathione peroxidase activity was measured by the procedure of Flohe and Gunzler (1984). Supernatant obtained after centrifuging 5% tissue homogenate for 10 min at 1500 × *g*, followed by 10,000 × *g* for 30 minutes at 4 °C, was used for GPx assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 ml of H_2O_2 (1 mM) and 0.3 ml of tissue supernatant. After incubation at 37 °C for 15 min, reaction was terminated by addition of 0.5 ml of 5% TCA. Tubes were centrifuged at 1500 × *g* for 5 min and supernatant was collected. 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of the reaction supernatant. After mixing well, absorbance was recorded at 420 nm.