NeuroToxicology 51 (2015) 184–191

Contents lists available at ScienceDirect

NeuroToxicology

Neurotransmitters and neuronal apoptotic cell death of chronically aluminum intoxicated Nile catfish (*Clarias gariepinus*) in response to ascorbic acid supplementation

Samah R. Khalil [a,*], Mohamed M.A. Hussein [b]

[a] Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Zagazig University, Egypt
[b] Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form 25 August 2015
Accepted 16 September 2015
Available online 13 October 2015

Keywords:
Ascorbic acid
Aluminum
Amino acid neurotransmitters
Apoptosis
Clarias gariepinus
Monoamine neurotransmitters

ABSTRACT

Few studies have been carried out to assess the neurotoxic effect of aluminum (Al) on the aquatic creatures. This study aims to evaluate the neurotoxic effects of long term Al exposure on the Nile catfish (*Clarias gariepinus*) and the potential ameliorative influence of ascorbic acid (ASA) over a 180 days exposure period. Forty eight Nile catfish were divided into four groups: control group, placed in clean water, ASA exposed group (5 mg/l), $AlCl_3$ received group (28.96 μg/l; 1/20 LC_{50}), and group received $AlCl_3$ concomitantly with ASA. Brain tissue was examined by using flow cytometry to monitor the apoptotic cell population, HPLC analysis for the quantitative estimation of brain monoamine neurotransmitters [serotonin (5-HT), dopamine (DA), norepinephrine (NE)]. The amino acid neurotransmitters [serum taurine, glycine, aspartate and glutamine and brain gamma aminobutyric acid (GABA)] levels were assessed, plus changes in brain tissue structure using light microscopy. The concentration of Al in both brain tissue and serum was determined by using atomic absorption spectrophotometery. The Al content in serum and brain tissue were both elevated and Al exposure induced an increase in the number of apoptotic cells, a marked reduction of the monoamine and amino acids neurotransmitters levels and changes in tissue morphology. ASA supplementation partially abolished the effects of AL on the reduced neurotransmitter, the degree of apoptosis and restored the morphological changes to the brain. Overall, our results indicate that, ASA is a promising neuroprotective agent against for Al-induced neurotoxicity in the Nile catfish.

1. Introduction

Aquatic systems around the world faced serious threats from anthropogenic contaminants such as trace metals, particularly in the developing countries like Egypt. The problems of the drainage canals have extremely been increased, resulting in the presence of high concentrations of such compounds in aquatic media, including various water bodies and sediment (Atli et al., 2006). Under certain environmental conditions, they might accumulate up to toxic concentrations causing ecological damage and affecting the health of aquatic habitats, particularly fish (Bai et al., 2011).

Some metals have been considered the potential contributing agents in the neurodegenerative disorders like Alzheimer disease (AD), and Parkinson's dementia (PD) via enhancing the degeneration of the neuronal cells (Zatta et al., 2003). Among those metals, Al(III), which accumulated in the animal and human body affecting their health. The mechanisms behind Al toxic insult to the brain are not clear and many hypotheses have been suggested. These hypotheses include exacerbation of oxidative stress (Moustafa et al., 2012), disruption of calcium homeostasis (Walton, 2012) and impairment of intracellular signal transduction pathways (Shafer and Mundy, 1995). Al induced neurotoxicity would certainly affect neurotransmission and related behavior and therefore changes in the neurotransmission would certainly affect such responses. This study focuses on the analysis of neurotransmitters, whereas neurotransmission is among the most specialized functions of nervous system.

Al is the most widely distributed metal in air, water and soil and is extensively used in modern daily life, it accounts for about 8% of the earth crust mass. Naturally, Al released into aquatic media as a result of soil erosion or mobilization from sediment, especially under acidic condition (Nayak, 2002), inducing damage that based

* Corresponding author at: Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt.
Tel.: +20 1063931398.
E-mail address: dr_samahkhalil@yahoo.com (S.R. Khalil).

http://dx.doi.org/10.1016/j.neuro.2015.09.008
0161-813X/

on the target organ, such as inflammation in the crayfish (Woodburn et al., 2011), respiratory dysfunction, ion regulation and osmoregulatory disruption in brown trout fish (Andrén and Rydin, 2012) as well as oxidative stress in lymphocytes of common carp (García-Medina et al., 2010).

Although the neurotoxic effects of Al for human either in vivo (Kawahara, 2005) or in vitro (Campbell et al., 2001) and murine (mammalian models) (Hussein et al., 2010; Moshtaghie et al., 2013) have been demonstrated, data concerning the potential neurotoxic impact of this metal on aquatic creatures particularly fish are still scarce.

Since the discovery of ascorbic acid (ASA), the number of its known biological and physiological functions is constantly expanding. All known functions of ASA are due to its action as an electron donor, in which the ability to donate one or two electrons makes it an excellent water-soluble reducing agent and donor antioxidant (Buettner and Schafer, 2006). The literature implicating ASA in the prevention of chronic and neurodegenerative diseases as AD remains controversial (Bowman, 2012). Under pro-oxidant conditions, it protects low density lipoprotein from oxidation and reduces harmful oxidants in the central nervous system. Among neuroprotective properties ASA has been shown to protect against; the seizures induced by Pilocarpine (Santos et al., 2009), restraint stress-induced memory deficits in wistar rat (Kumar et al., 2009a), diazepam and scopolamine-induced memory impairment (Parle and Dhingra, 2003) as well as propionic acid-induced cognitive deficits in rat (Pettenuzzo et al., 2002).

Clarias gariepinus, an omnivorous fish, is one of the important freshwater fish in Egypt and many other countries. They are found in South Africa and northern Africa, Europe, the Middle East, and in parts of Asia. It has a high growth rate and very adaptive to extreme environmental conditions, it has been proposed as a very good food source for humans as it has a high biological value (low cholesterol content and one of the safest sources of animal protein). This species has shown considerable potential for use in an intensive aquaculture. Because of this, this species involved in extensive diet experiments concerning with finding of the best diet for optimal yield. This species is also important in nutrient recycling in conjunction with rice fields, which increases rice yields (Ali and Jauncey, 2004; Na-Nakorn et al., 2004).

In Egypt, this species is frequently consumed by humans and is often cultured in water bodies contaminated by diverse xenobiotics including heavy metals. One such case is that of Al in Delta Rosetta branch, where high concentrations of this metal have been detected in both surface water and bed sediment with concentrations of <0.01–0.36 mg/l and 10.1–40.4 g/kg, respectively (El Bouraie et al., 2010). Also, it detected in different regions such as the Nile Delta and the Nile River basin, the Mediterranean sea Coast and the Gulf of Suez (Red Sea) (Hamed and Emara, 2006; El Nemr et al., 2007a, 2012; Khaled et al., 2012; Khalil et al., 2015). In this context, this study was interested in evaluating the neurotoxic effect of chronic (long-term) exposure to Al through assessment of neurotransmitters impairments, apoptosis and neuropathological alterations in Nile catfish (*C. gariepinus*), as well as the probable detoxifying efficacy of ASA in the prevention of these neurotoxic impairments.

2. Materials and methods

2.1. Tested compounds and chemicals

Aluminum chloride ($AlCl_3{\cdot}6H_2O$) in the form of white or pale yellow crystalline powder with a molar mass of 241.45 g/mol (hexahydrate) was purchased from Oxford Lab. Chem, India. Ascorbic acid, easily soluble in water were procured from SD Fine Chem Ltd., Mumbai, India with a molar mass 176.13 g/mol. All other reagents and chemicals used were of analytical grade purchased from Sigma-Aldrich Co., St. Louis, MO, USA.

2.2. Fish and experimental design

Forty eight Nile catfish (140–160 g, 20–23 cm in total length) obtained from El-Abassa Fish Hatchery Farm, Sharkia Governorate, Egypt. They were maintained in cement ponds at the Fish Research Center of Faculty of Vet. Med., Zagazig University and acclimatized to laboratory conditions in glass tanks for two weeks before exposure. Experimental tanks contained 96 l of dechlorinated and gently aerated tap water. All the aquaria were kept under the same conditions of water temperature (25 ± 1.02 °C), pH (6.9 ± 0.1), dissolved oxygen (7.4 ± 0.34 mg/l) and ammonia (0.035 ± 0.01 mg/l) with a controlled photoperiod (10 h light:14 h dark) in the laboratory. They were fed once daily (at 10:00 AM) with a normal commercial fish dry pellets at a rate of approximately 3% of their body weight allover the duration of the experiment.

The Nile catfish were randomly assigned into four equal groups, each containing 12 fish, Water control group: fish were placed in clean tap water. ASA control group: fish were received 5 mg/l ASA for 6 months (Kumar et al., 2009b). $AlCl_3$ group: fish were exposed to $AlCl_3$ at a dose of 1/20 LC_{50} value (28.96 μg/l) for 6 months (96-h LC_{50} value of $AlCl_3$ was calculated as 568.34 μg/l for Nile catfish) (Hamed, 2012) and $AlCl_3$/ASA group: fish were exposed to 1/20 LC_{50} value (28.96 μg/l) for 6 months and ASA (5 mg/l) which simultaneously administered for the same duration.

Throughout the experiment, control and experimental fish were maintained in static renewal conditions, where water, $AlCl_3$ and ascorbic acid were completely replaced every 48 h by transferring the fish to another aquarium. The experimental protocol was approved by the Ethics Committee on the Use and Care of Animals (EAURC), Cairo University, Egypt.

2.3. Serum collection and brain tissue preparation

After 6 months of exposure, blood samples were collected from the caudal vein of individual fish, centrifuged at 3.000 rpm for 10 min to obtain serum samples which were stored at −20 °C until the analysis of amino acid neurotransmitters and determination of Al concentration. Following sacrificing experimental and control fish. Brain was immediately dissected and washed with ice cold phosphate buffered saline (PBS), then assigned into 3 parts, stored at −20 °C until the flow cytometric analysis, estimation of monoamine neurotransmitter and Al concentration.

2.4. Biochemical and Al level analysis

2.4.1. Assessment of Al concentration in serum and brain tissue

For the determination of Al concentration in serum and brain tissue, 1 g, ml of brain tissue, serum samples respectively were initially digested with 4 ml of nitric/perchloric acid (volume ratio is 4:1) for over 24 h at room temperature, followed by heating at 110 °C for 2 h in water bath. When the mixture became transparent and the color was yellowish brown, the digestion was over, then allowed to cool at room temperature, filtered, and diluted with deionized water prior to analysis (Julshman, 1983). Using pure certified metals and Merck grade HNO_3 (65%) and H_2O_2 (30%), standard solutions were prepared at different ppm levels to construct a calibration curve. A standard reference material (NBS-bovine liver, No.1577 a) from the National Institute of Standards and Technology (NIST) was used to control the precision and the accuracy of the analytical procedure. Comparisons of the certified values for each metal with the analyses of the reference material revealed recovery rate of 96% for Al. Al concentrations were