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Original article

Protective effects of deferiprone and desferrioxamine in brain tissue of aluminum intoxicated mice: An FTIR study

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ABSTRACT

The present study was designed to study aluminum chloride which caused marked alterations in biochemical parameters such as glutathione peroxidase, catalase, superoxide dismutase, and TBARS in brain tissues of mice. Fourier transform infrared spectroscopy spectra reflect the alterations on major biochemical constituents in brain tissues of mice such as proteins, lipids and nucleic acids due to the overproduction of ROS. Furthermore, administration of deferiprone and deferoxamine significantly improved the level of protein and shifted back the peak positions of amide I and II to near control values indicating tau protein, β-amyloid, amyloid plaques and neurofibrillary tangles decreased, consequently protected from Alzheimer's disease and other major risk factor of many neuronal dysfunctions in brain tissues. Therefore, aluminum toxicity is a widespread crisis to all living organisms, including both flora and fauna. Furthermore, it causes widespread degradation of the environment and health. Therefore, the present investigation suggested that DFO and DFP are efficient chelators for aluminum poisoning and they reduced the aluminum concentration.

1. Introduction

Aluminum is the most abundant metal and the third most abundant element in the earth's crust, after oxygen and silicon [1]. The main entry sites of Al into the body are the gastrointestinal, respiratory tract and accumulation in several tissues, like spleen, lungs, liver, kidneys, heart, bone and brain [2]. High brain levels of Al induce cognitive deficiency and dementia and, thus, Al is a widely accepted neurotoxin [3]. Moreover, it can be degenerate of nerve cells in the brain of humans and experimental animals. Absorption/accumulation of Al in humans can occur via the diet, drinking water, vaccines, antacids, parenteral fluids, inhaled fumes and particles from occupational exposures [4]. Al has been proposed as an environmental factor that may contribute to some neurodegenerative diseases and affect several enzymes and other biomolecules relevant to Alzheimer's disease (AD) [5]. Aluminum is known to potentiate iron-related reactive oxygen species (ROS) formation in isolated systems [6]. For this reason, the current study used groups of mice treated with aluminum and combined therapy of deferiprone with deferoxamine in order to determine whether aluminum enhanced ROS production in brain as well as determined the antidotes capacity of both chelators. Al chloride was chosen over other Al species because the stomach already contains and utilizes chloride, so this form of Al can be introduced with minimal change to gastric fluid composition.

The oral administration of deferiprone was an effective treatment and it determines if the chelating agent deferiprone could mobilize aluminum from tissues and increase aluminum elimination [7]. Deferiprone is more effective, water soluble, less toxic than DFO and can be given orally. Aluminum chelators DFO and DFP should be capable to mobilize aluminum and to reduce body burden, so repealing both encephalopathy and osteomalacia. The first aluminum chelator introduced in clinical practice for aluminum-related osteomalacia was deferoxamine [8].

Deferiprone does not manifest the same pattern of toxicity as deferoxamine due to the different chemical properties of these chelators. DFP and DFO simultaneous administration and the aggressive pharmacologic treatment were essential to induce a regression of brain dysfunction in a very short time, which was associated with an improvement in clinical status [9]. Previously, iron chelation therapy is attained by the use of subcutaneous deferoxamine pumps; or more recently, through the use of the oral iron chelators deferiprone and deferoxamine [10]. Chelation therapy which suggests that surely translated into prolonged survival and enhanced quality of life for patients with brain diseases. DFO and DFP compounds are usually employed in iron accumulation but there are chemical and physical similarities between aluminum and iron (charge, ionic radius and protein binding)

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and both have been used in case of aluminum accumulation [11].

Fourier transform infrared spectroscopy (FTIR) is a powerful technique, which has been widely used in biophysical and biochemical research, demonstrated to provide sensitive and precise measurement of biochemical changes in biological cells and tissues [12]. The frequency shifts demonstrate the molecular alteration of macromolecules such as protein, lipid, carbohydrate, and nucleic acid. Previously, it was reported that the relationship between human and mice brain shares numerous features of brain organization and behavioral responses to many pharmacological agents [13]. Moreover, chelation therapy is one of the most effective methods to remove toxic elements from a biological system [14]. Therefore, the main objective of this study was to use this molecular fingerprinting approach to investigate the effects of deferiprone and desferrioxamine on metabolic alterations that occur in brain tissues of aluminum-intoxicated mice.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (*Mus musculus*), weighing 25 ± 2 g, were procured from the central animal house, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, and were maintained in an air-conditioned room (25 ± 1 °C) with a 12-h light/12-h dark cycle. Feed and water were provided ad libitum to all the animals. The study was approved by the Institutional Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA, Proposal number: 851), Annamalai University, Annamalai Nagar-608002, Tamilnadu, India.

2.2. Test chemicals

Deferroxamine (Desferal) and deferiprone (DFP) were purchased from Novartis and Sigma Aldrich, Chemicals Limited, Mumbai, India. All other chemicals used in this study were of highest analytical grade obtained from Sisco Research Laboratories and Himedia, Mumbai, India.

2.3. Treatment schedule

Mice were randomly allocated in four different groups. Each group contained 12 animals. Group I served as control and was fed on standard animal chow and water ad libitum, received an i.p. injection of 0.9% saline and deionized water by gavage. Animals in groups II, III and IV were administered aluminum in the form of aluminum chloride (100 mg/kg b.wt./day) orally for a period of 16 weeks. Group III mice were treated with DFP (p.o.) at the dose of 0.72 mmol/kg and Group IV were treated with DFO + DFP (i.p.) at the dose of 0.89 mmol/kg dose, half an hour after a single i.p. administration of 100 mg Al/kg body weight in the form of aluminum chloride for five consecutive days. Aluminum chloride was dissolved in normal drinking water and was given by oral gavage. Twenty-four hours after the last dose of aluminum chelators, the animals were sacrificed under ether anesthesia by cutting jugular veins. The brain were excised, weighed immediately then stored at −80 °C until analysis.

2.4. Sample preparation

For FTIR analysis, the samples were mixed with KBr at ratio of 1:100. The mixture was then subjected to a pressure of 1100 kg/cm^2 to produce KBr pellets for use in FTIR spectrometer. Pellets of the same thickness were prepared by taking the same amount of sample and applying the same pressure. Consequently, in the current study, it was possible to directly relate the intensities of the absorption bands to the quantity of the corresponding functional groups.

2.5. FTIR spectra and data analysis

FTIR spectra of the region 4000–400 cm^{-1} were recorded at the temperature of 25 ± 10 °C on a Nicolet Avatar 360 FTIR spectrometer equipped with an air cooled DTGS (deuterated triglycine sulphate) and purged with nitrogen. Each sample was scanned with three different pellets under identical conditions. These replicates were averaged and then used. The spectra were analyzed using ORIGIN 6.0 software (OriginLab Corporation, Massachusetts, USA).

2.6. Biochemical analysis

The concentration of thiobarbituric acid reactive substances (TBARS) was estimated by the method of Niehaus and Samuelson [15]. The activities of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assayed by the methods of Kakkar [16], Sinha [17] and Rotruck [18] respectively.

2.7. Histopathological examination of brain tissues

Mice (six mice/treatment group) at the end of experiment were dissected for histology. Removed brain samples were cleared of blood and immediately placed in a neutral buffered solution of 10% formalin for 24 h. Sliced into 5 μm thickness and then placed onto glass slides. The sections were stained with haematoxylin and eosin staining (H&E) and examined by light microscopy which have been used to visualize changes in tissue structures. The cross-sectional area (CSA) of brain was evaluated from photographs of whole tissue sections taken at 40× magnification and scanned, digitized and analyzed by computer, using the Adobe Photoshop Imaging Program (Adobe System Incorporation).

2.8. Statistical analysis

Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 16 (SPSS, Chicago, IL). Probability level (*P*-value) of less than 0.05 was considered statistically significant.

3. Results

FTIR is a non-perturbing rapid technique giving information on several biomolecules, such as DNA, RNA, proteins, carbohydrates, lipids in biological tissues and cell. FTIR spectroscopy basically deals with the mid-infrared region 4000–500 cm^{-1} (2.5–25 μm wavelength) which is the most informative for biosamples since these contain mainly organic compounds [19] and provide information about chemical bonding properties that characterize biochemical functional components in complex matrices and allow for qualitative identification and quantitative estimations [20].

The list of absorption peak assignments belonging to lipids, proteins, polysaccharides, carbohydrates and nucleic acids for various functional groups and representative infrared spectrum in the region between 4000 and 400 cm^{-1} are presented in Table 1 and Fig. 1 respectively. For our convenient, Fig. 2 spectra were analyzed in three major distinct regions: 3750–2700 cm^{-1} (I), 1800–1350 cm^{-1} (II) and 1300–400 cm^{-1} (III). Curve fitting procedure was not applied because the bands were clearly resolved [21,22]. The band area values, band area ratios and biochemical analysis are presented in Tables 2–4 respectively.