Journal of Inorganic Biochemistry 139 (2014) 57–64

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Occupational exposure to aluminum and its amyloidogenic link with cognitive functions

N.H. Zawilla [a], F.M. Taha [b,*], N.A. Kishk [c], S.A. Farahat [a], M. Farghaly [c], M. Hussein [d]

[a] Department of Occupational & Environmental Medicine, Faculty of Medicine, Cairo University, Egypt
[b] Department of Medical Biochemistry, Faculty of Medicine, Cairo University, Egypt
[c] Department of Neurology, Faculty of Medicine, Cairo University, Egypt
[d] Department of Neurology, Faculty of Medicine, Bani-suef University, Egypt

ARTICLE INFO

Article history:
Received 7 March 2014
Received in revised form 29 May 2014
Accepted 2 June 2014
Available online 11 June 2014

Keywords:
Aluminum
Occupational exposure
Amyloidogenesis
Amyloid precursor protein
Cathepsin D activity
ACE-R test

ABSTRACT

As many other metals, aluminum is a widely recognized neurotoxicant and its link with neurodegenerative disorders has been the subject of scientific debate. One proposal focuses on amyloid β deposition (amyloidogenesis) as the key player in triggering neuronal dysfunction the so-called amyloid cascade hypothesis. We undertook this study first to investigate the cognition status of workers exposed to Al dust in an Al factory in Southern Cairo, second, to evaluate serum amyloid precursor protein (APP) and cathepsin D (CD) enzyme activity to study the possible role of Al in amyloidogenesis, and finally to explore the relation between these potential biomarkers and cognitive functions. The study was conducted on 54 exposed workers and 51 matched controls. They were subjected to questionnaire, neurological examination and a cognitive test battery, Addenbrooke's Cognitive Examination — Revised (ACE-R). Serum Al, APP and CD enzyme activity were measured. A significant increase of serum Al was found in the exposed workers with an associated increase in serum APP and decrement in CD activity. The exposed workers displayed poor performance on the ACE-R test. No significant correlation was detected between ACE-R test total score and either APP or CD activity. We concluded that occupational exposure to Al is associated with cognitive impairment. The effect of occupational Al exposure on the serum levels of APP and CD activity may be regarded as a possible mechanism of Al in amyloidogenesis. However, our findings do not support the utility of serum APP and CD activity as screening markers for early or preclinical cognitive impairment.

1. Introduction

Aluminum (Al) is a ubiquitous element in nature. It is the third most common element in the earth's crust and causes unavoidable environmental exposure. Since the metal has no essential function in the mammalian organism but is toxic under special circumstances, it can be regarded as a harmful contaminant for humans. However based upon its physical and chemical properties Al has gained wide industrial and commercial importance [1]. Occupational exposure to Al occurs during refining of the primary metal and in secondary industries that use Al products. Secondary Al smelters involve recycling Al products and scrap [2]. Depending on the process and impurities contained in scrap Al, ambient air in Al smelters may contain a multitude of compounds in addition to Al [3].

Al is a well-established neurotoxicant and is suspected to be linked with various neurodegenerative diseases including Alzheimer's disease (AD) [1]. The etiological factors of AD are not clearly known, although several hypotheses had been studied and some were proved including genetics, head trauma, oxidative stress, infectious agents, and environmental factors [4].

Alzheimer's disease is a progressive neurodegenerative disorder and the most common form of dementia. The pathological hallmarks of AD are the deposition of extracellular senile plaques, intracellular neurofibrillary tangles (NFTs), and the selective loss of synapses and neurons in the hippocampal and cerebral cortical regions [5]. The major component of NFTs is the phosphorylated tau protein, while senile plaques are largely comprised of amyloid beta peptide (AβP). AβP is generated via sequential proteolytic cleavage of amyloid precursor protein (APP), mainly through β-secretase and γ-secretase enzymes [6].

The scientific literature contains numerous studies that discuss the link between Al and various neurodegenerative disorders [7–9]. The possible relation between Al and AD was hypothesized based on epidemiological studies relating the Al content of drinking water with increasing incidence of dementia and AD [10,11]. Further, Al was suggested to be the cause of dialysis-associated encephalopathy (DAE) [12]. However, the DAE pathology was found to be clearly different from AD pathology through histological analyses of dialysis patients by light microscopy [13]. Worldwide, the last outbreak of Al toxicosis was reported in 2001 in Curaçao where 10 patients died. The incident

* Corresponding author. Tel.: +20 25323679 (office); fax: +20 25323679.
E-mail address: fatma.taha@kasralainy.edu.eg (F.M. Taha).

http://dx.doi.org/10.1016/j.jinorgbio.2014.06.003
0162-0134/

was associated with a cement mortar distribution pipe from which Al and calcium leached into the water used to prepare the dialysate [14]. In Canada, Neri and Hewitt reported that Al concentration over 0.2 mg/L in water may increase the incidence rate of AD (odds ratio = 1.46) [15]. However, fluorine content, rather than Al, was found to be more correlated with AD [16]. Similarly no significant relationship was obtained for Al effect in drinking water in several other studies [17,18].

Regarding occupational Al exposure, studies did not produce consistent results regarding cognitive impairment. Polizzi et al. found a negative relationship between serum Al levels and mini-mental state exam (MMSE) and the clock drawing test scores; there was a positive relationship between serum Al levels and both test times [19]. Contradictory findings were deduced by other studies which have raised the question as to whether the metal may play a role in these neurological disorders [20–22].

Aβ peptide and APP accumulation in the brain are the key factors in initiation and progression of amyloidogenesis in neurodegenerative diseases. APP gene was one of the seven genes found to be significantly up-regulated by Al ions in human neural cells [23]. The best model for the diagnosis of incipient AD was cerebrospinal fluid (CSF) sAPP and tau, with a sensitivity of 95.20% and a specificity of 81.20%. Indeed the diagnostic utility of these markers was supported [24]. However several other studies did not support such conclusion [25,26]. Research data suggest that Al may modulate the expression and processing of APP [27,28]. Indeed chronic oral ingestion of Al gradually accumulated in brain regions and was sufficient to increase APP levels and launch the cascade that resulted in the formation of amyloid plaques in the brain [29].

Peripheral changes of APP cleavage products may be more closely related to cerebral changes due to AD than to a pathological response in the periphery [30]. Increased concentrations of full-length APP protein in blood platelets and APP mRNA levels in blood mononuclear cells have been reported in AD [31,32]. Moreover plasma APP levels mirrored the changes observed with CSF levels in AD [33,34].

Cathepsin D (CD) is an active acid protease that is initially produced as a nonfunctional enzyme in the trans Golgi network in the rough endoplasmic reticulum and undergoes various proteolytic transformations until it reaches its targeted intracellular vesicular structure where it is involved in intracellular protein breakdown. The functions of CD are to hydrolyze APP protein and to clear Aβ peptide from the central nervous system. As such, CD might involve in the pathogenesis of AD [35,36]. Indeed variants of CD gene can impede the functions of proteolytic degradation, thus increasing the risk of AD. This gene polymorphism was significantly associated with the general intelligence of healthy elderly [37], with the T allele (CD-C/T gene) considered as a high-risk factor for developing AD [38,39]. However these results were not replicated in many other studies [40–43].

One of the mechanisms by which Al causes its neurotoxicant effect is through inhibition of protein functions and enzymatic activities. Studies using neuroblastoma cells or rat cortical neurons have described endocytosis of AL with its accumulation inside lysosomes [44,45]. Further, Aβ peptides were accumulated and degraded in the lysosomes of the microglia by CD in lysosomes. Nakanishi described the pathological roles of neuronal and microglial cathepsins in brain aging and age related diseases [46]. The potential of Al to interact and disrupt Aβ peptide catabolism via the inhibition of its proteolytic degradation by CD was demonstrated [47].

Currently neurodegenerative disorders are diagnosed by a multitasking process involving neuropsychological tests, imaging and CSF assessment. CSF biomarkers include total tau protein (t-tau), phosphorylated tau (p-tau), and the 42 amino acid isoform of Aβ (Aβ42) [48]. However, CSF biomarkers, though clinically validated, are not routinely used and are not ideal in a perspective of mass-screening in the general or at risk population. To this respect, blood-derived circulating biomarkers would be a better solution. Possibly because at peripheral level, the clinical picture is variable, blood biomarkers have not yielded consistent, easily reproducible or sensitive levels for diagnosis, evaluation of disease progression, or treatment effects [48,49]. However promising results were presented by Ray et al. who developed from a panel of 18 plasma circulating molecules an algorithm for discriminating AD patients from controls and progression of mild cognitive impairment (MCI) subjects to AD [50].

Accordingly we undertook this study first to investigate the cognition status among workers occupationally exposed to Al dust in an aluminum smelter and production lines, second, to evaluate the serum APP and CD enzyme activity in exposed workers as a possible mechanism of Al role in amyloidogenesis, and finally to explore the relation between these potential biomarkers and cognitive functions and their possible utility as screening tools for early cognitive impairment in Al exposed workers.

2. Materials and methods

2.1. Materials

This cross sectional study was conducted on the whole production working population (n = 54) in one of the major aluminum factories in Helwan area, Southern Cairo. The factory is producing anodized and electro-static powder coated aluminum profiles for tubes, transportation systems including busses and trucks and many other applications. The factory constitutes many departments namely, extrusion presses, anodizing, electrostatic powder coating and Al smelters (primary and secondary).

All eligible employees were invited to participate in the study. Eligibility criteria for exposed workers included regular employment in the factory for at least the preceding 5 years. Those who met the criteria for inclusion were the exposed population and they comprised 54 workers (23 from the Al smelters and 31 from other production lines in the factory). Fifty one controls were recruited from workers in simple low rank administrative jobs that did not carry the risk of exposure to Al (porters, clerks, security personnel and switch operators). The controls were chosen so as to be matched with exposed workers regarding age, sex, educational level and smoking status.

Exclusion criteria for both the exposed and control workers were: any history of alcohol intake or drug abuse and regular intake of medications for hyperacidity as they have high Al content, and any condition that may cause cognitive impairment including liver, kidney, cerebrovascular diseases and uncontrolled diabetes. Workers with marked poor vision were excluded to avoid poor performance in the Addenbrooke's Cognitive Examination.

2.1.1. Ethical considerations

All participants were literate. Subjects were treated according to the Helsinki Declaration of biomedical ethics. Informed consent was obtained from all subjects after proper orientation regarding the objectives of the study, data confidentiality and the impact of the study. The study was approved by the Research Ethics Committee of Faculty of Medicine, Cairo University (N-54-2012).

2.2. Methods

2.2.1. A specially designed questionnaire

A specially designed questionnaire was administered during an in-person interview. We investigated confounding variables considered to be possible risk factors for cognitive impairment. The questionnaire consisted of detailed questions regarding self-reported illness, health and well-being, and life-style habits such as smoking, alcohol consumption, medications, occupation and work environment. Medical history included major psychiatric illnesses, chronic neurological diseases such as cerebrovascular stroke, Parkinsonism, epilepsy, intracranial neoplasms, major medical diseases such as renal, hepatic, metabolic