Journal of Inorganic Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Is exposure to aluminium adjuvants associated with social impairments in mice? A pilot study

Sneha K.S. Sheth, Yongling Li, Christopher A. Shaw*

University of British Columbia, Vancouver, Canada

ARTICLE INFO

Keywords:
Aluminium
Adjuvants
Vaccines
Neurotoxicity
Social behaviours
Autism spectrum disorders

ABSTRACT

Background: Our group has shown that significant correlations exist between rates of Autism Spectrum Disorder (ASD) and total aluminum adjuvants given to children through vaccines in several Western countries. These correlations satisfied eight out of nine Hill criteria for causality. Experimental studies have demonstrated a range of behavioural abnormalities in young mice after postnatal exposure to aluminium. To build on our previous work, the current study will investigate the effect of aluminium adjuvants on social behaviour in mice. Anomalies in social interaction are a key characteristic of those with ASD.

Methods: Neonatal CD-1 mice pups were injected with either a total of 550 μg of aluminum hydroxide gel (experimental group) or saline (control) spread out during the first two weeks of postnatal life. The mice were then subjected to behavioural tests for social interest and social novelty at postnatal week 8, 17 and 29. p-Values were calculated using the Mann-Whitney and Kruskal Wallis tests.

Results: Aluminum injected mice showed diminished social interest compared to controls at week 8 ($p = 0.016$) and 17 ($p = 0.012$). They also demonstrated abnormal social novelty from controls at week 8 ($p = 0.002$) and week 29 ($p = 0.042$).

Conclusion: This is the first experimental study, to our knowledge, to demonstrate that aluminum adjuvants can impair social behaviour if applied in the early period of postnatal development. The study, however, is insufficient to make any assertive claims about the link between aluminium adjuvants and ASD in humans.

1. Introduction

Aluminium (Al) is the most abundant metal found in the Earth's crust, however, it has no known role in any biological processes and is thus considered to be non-essential for life [1]. Given the ubiquitous presence of aluminium in the modern environment, chronic exposure to aluminium is unavoidable.

Aluminium exposure commonly occurs through products such as deodorants, cosmetics, dyes, processed foods, antacids, medicinal pills, drinking water, and vaccine adjuvants [2] [3] [4]. Adjuvants are agents added to vaccines that act through various immune-stimulating mechanisms in order to increase the specific immune response or responses to infectious antigens [5].

Several studies have repeatedly confirmed that accumulation of aluminium from any source can produce neurotoxicity in the central nervous system (CNS) [6–16]. Aluminium has been etiologically linked with several diseases such as Alzheimer's disease, amyotrophic lateral sclerosis, dialysis encephalopathy, Parkinson's disease, Gulf-War syndrome, epilepsy and multiple sclerosis [18–20]. Aluminium adjuvants, in particular, have been linked with a variety of neuromuscular and multiple organ system dysfunctions, including macrophagic myofasciitis (MMF), and autoimmune/inflammatory syndrome induced by adjuvants (ASIA) [21,22].

One of the factors that influences the toxic potential of aluminum is the route of administration [23]. For ingested aluminium, the poor solubility of aluminum compounds allows for its effective excretion by the kidneys; with only about 0.25% of the ionic aluminum getting absorbed into the blood for those with normal kidney function [24,25]. Sweat is another major route of aluminum excretion [26]. However, almost 100% of the intramuscularly injected aluminum (as in vaccine adjuvants) is absorbed into the systemic circulation and travels to different sites in the body such as the brain, joints and the spleen where it accumulates and is retained for years post-vaccination [8,9,25]. Moreover, although the half-life of enterally administered aluminum is short (approximately 24 h), adjuvanted aluminum takes much longer to be eliminated because of its exceptional affinity for the various antigens. The latter is the very feature that allows it to activate an elevated immune response and thus act desirable adjuvant. Two other key

* Corresponding author.
E-mail address: snehasheth@psych.ubc.ca (S.K.S. Sheth).

https://doi.org/10.1016/j.jinorgbio.2017.11.012
Received 1 July 2017; Received in revised form 3 November 2017; Accepted 17 November 2017

aspects to keep in mind while addressing the question of toxicity are: (1) the aluminum dose in a given duration; for instance, the dose of aluminum in the hepatitis B vaccine which contains the lowest content of aluminum (250 μg) is five times that absorbed through 6 months of breastfeeding (55 μg) [27], and (2) the stage of neurodevelopment of the person being vaccinated. For example, an infant in the United States, in its first two years, usually receives 27 vaccines as part of the routine pediatric vaccination schedule; many of which contain aluminum adjuvants. This is a crucial period for major neurodevelopmental processes in an infant's brain, including the onset of synaptogenesis and extensive pruning of excessive synapses, during which the brain is highly susceptible to neurotoxic insults.

Aluminum has many effects on both the immune and central nervous systems. Effects of aluminium's neuro- and immuno-toxicity include impairment of neurotransmission and synaptic activity, disruption of the blood-brain barrier, microglial activation and brain inflammation, impairment of brain-specific gene transcription, neurite damage, amyloidosis and impairment of genetic resistance towards autoimmunity in both adults and infants [20].

Many of the aforementioned characteristics associated with neurotoxicity have also been observed in those with autism spectrum disorder (ASD). ASD is a neurodevelopmental disorder with the most recent prevalence reported to be at 1:68 in the United States [28], about 2000 times that before 1980 when it was a 'rare' disorder with a low prevalence that was relatively stable [20]. A sudden exponential rise in the prevalence of ASD cannot be explained through genetics alone or even a change in diagnostic criteria as, in many ways, the diagnostic criteria have become more stringent [29]. Despite evidence of genetic predispositions, the pathogenesis of ASD is yet unknown. Several studies have investigated the possibility of an environmental trigger, interacting with a set of susceptible genes, leading to the phenotype of ASD [30].

There has been considerable speculation on the role of vaccines in the contribution of the rising prevalence of ASD. A study by our group has shown a strong correlation between the rising prevalence of ASD and an increased aluminium dose through vaccine adjuvants given during early postnatal life [31]. However, ecological studies are unable to establish causality and are primarily aimed at generating valid hypothesis that can be examined by further experiments.

Another study conducted by our group has shown anomalies in behavioural outcomes in mice injected with aluminium as per the US pediatric vaccination schedule [32]. The current study has been designed to build on previous work by testing for behavioural deficits specific to a core symptom of ASD, namely, deficits in social behaviour.

2. Methods and materials

2.1. Aluminium adjuvant

Alhydrogel®, an aluminium hydroxide ($Al(OH)_3$) wet gel suspension, was used as a source of aluminium hydroxide. Alhydrogel™ 2% is a trademark of Brenntag Biosector and was purchased from INVIVOGEN.

2.2. Dosage and administration

The aluminium injection schedule in our study was intended to mimic the 2010 US pediatric vaccination schedule to maintain consistency with our previous work [31,32]. The approximate amount of aluminum in all those pediatric vaccines containing aluminium adjuvants (Table 1) at different ages in preschool children, was adapted from our previous study which found a strong correlation between prevalence of ASD and the exposure to aluminium from pediatric vaccination schedules.

As an extension of our previous work, the current study focused on the effects of aluminium on one key characterizing feature of ASD, namely anomalous social interaction. To investigate this, we have attempted to mimic the Al load from the US pediatric schedule as closely as practically possible, in CD-1 mice (Table 2) in a similar manner as done in our previous study [11]. For this purpose, new born mice pups were divided into two groups, Al injected ("Al") and saline controls ("Control"), consisting of 28 and 23 animals respectively. The litters after birth were equally and randomly divided into Al and control groups, both containing an equal number of males and females. The dosage of Al adjuvant injected in mice was approximately equivalent (μg/kg) to Al exposure through pediatric vaccines in children (Table 1).

Mice were weaned when they were sexually mature at 5–6 weeks of age. Since most pediatric vaccinations are given to children before the

Table 1
The following table displays the approximate total body burden of aluminum in preschool children from pediatric vaccines (in μg) at different ages as per the 2010 U.S. vaccination schedule [11]. The approximate equivalent amount of aluminum injected in CD-1 mice (according to the schedule in Table 2) is shown in bold text.

Vaccine	Birth	2 months	4 months	6 months	15 months	2 years	6 years
Hep B	250	250		250			
DPT[a]		375	375	375	375		375
Haemophilus influenza type b[b]		112.5	112.5	112.5	112.5		
Pneumococcal		125	125	125	125		
Hep A					250	250	
Total Al (μg)	250	862.5	612.5	862.5	862.5	250	375
Total Al (μg/kg bw)	73.5	172.5	107.5	113.5	78.4	19.8	19.3
Total Al (μg/kg bw) injected into neonatal CD-1 mouse	–	**170**	**150**	**110**	**80**	**20**	**20**

*Note:*Table 1 Adapted, with permission, from Shaw et. al [11]
[a] Mean value from three different brands of DTaP (Infanrix, Daptacel, Tripedia).
[b] Mean value from two different brands of Hib (PedVax and Hiberix).

Table 2
Dosage and schedule of aluminum hydroxide or saline injections in treated mice and control mice.

Treatment group	Amount of Al/saline injected each day (μg/kg bw)						Total Al or saline injected (μg/kg bw)
	PND 2	PND 3	PND 5	PND 9	PND 12	PND 16	
Al or saline	170	150	110	80	20	20	550