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Research report

Accumulation of aluminum by primary cultured astrocytes from aluminum amino acid complex and its apoptotic effect

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Abstract

Aluminum salts or doses that are unlikely in the human system have been employed in toxicity studies and much attention had been focused on the secondary target (neurons) of its toxicity rather than the primary target (astroglia). In order to address these issues, we have investigated the uptake and apoptotic effects of aluminum amino acid complex on primary cultured astrocytes because these are fundamental in understanding the mechanism of aluminum neurotoxicity. Aluminum solubilized by various amino acids was differentially internalized by astrocytes (glycine>serine≫glutamine≫glutamate), but aluminum was not internalized from citrate complex following 24 h of exposure. Inhibition of glutamine synthetase, by methionine sulfoximine (MSO), enhanced the uptake of aluminum from various amino acid complexes within 8 h except from glutamine complex. Blockade of selective GLT-1 (EAAT2) and GlyT1, as well as nonspecific transporters, did not inhibit or had no effect on uptake of aluminum in complex with the corresponding amino acids. Ouabain also failed to inhibit uptake of aluminum complexed with glycine. Pulse exposure to aluminum glycinate in the absence or presence of MSO caused apoptosis in over 25% of primary cultured astrocytes, and apoptotic features such as chromatin condensation and fragmentation became evident as early as 3 days of culture in normal medium. Lower doses (as low as 0.0125 mM) also caused apoptosis. The present findings demonstrate that aluminum solubilized by amino acids, particularly glycine, could serve as better candidate for neurotoxicity studies. Citrate may be a chelator of aluminum rather than a candidate for its cellular uptake. Amino acid transporters may not participate in the uptake of aluminum solubilized by their substrates. Another pathway of aluminum internalization may be implicated in addition to passive diffusion but may not require energy in form of Na^+/K^+-ATPase. Impaired astrocyes' metabolism can aggravate their accumulation of aluminum and aluminum can compromise astrocytes via apoptosis. Thus, loss of astrocytic regulatory and supportive roles in the central nervous system (CNS) may be responsible for neurodegeneration observed in Alzheimer's disease.

Theme: Disorders of the nervous system
Topic: Neurotoxicology

Keywords: Aluminum amino acid complex; Internalized aluminum; Metabolic perturbation; Amino acid transporter; Apoptosis; Astroglial culture

1. Introduction

The recognition of aluminum as a neurotoxic agent in animal dates back to over 100 years [25], but the idea that aluminum may be involved in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease (AD), was first suggested about four decades ago in the report of Klatzo et al. [39]. About a decade following this report, Crapper et al. [18] corroborated the findings of Klatzo et al. Alfrey et al. [5] also reported their findings, implicating aluminum in the etiology of dialysis encephalopathy. Since these times, the public has been besieged by conflicting reports supporting, refuting, or equivocal on those claims. Aluminum has also been implicated in several other neurological and non-neurological disorders. However, the existence of causal relationship between aluminum and neurodegenerative disorders, such as AD, remains

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unclear or controversial. Nevertheless, it is known that as over 95% of cases with AD are sporadic, some environmental factors are expected to be etiological [47]. Aluminum is an environmental factor, which is a ubiquitous element used extensively in contemporary life. Until the controversial role of aluminum as an environmental factor in the pathogenesis of neurodegenerative diseases is well resolved, therefore, it will continue to be a subject of man's curiosity.

Recent findings have implicated astrocytes as the principal target for aluminum toxic action [13,31,72,74]. Thus, the primary culture of astrocytes would provide a good model for evaluating neurotoxic injury. Unfortunately, the majority of works on the toxic effects of aluminum has involved an examination of the direct effects of aluminum on neuronal cells, while works on the toxic effects of aluminum on astrocytes are lagging behind. However, the astroglial environment of neurons provides metabolic and trophic support, and contributes to local modulation of synaptic efficacy at excitatory inputs by controlling glutamate clearance and represents an important regulator of glutamatergic communication between dependent synapses by setting the parameters of diffusion in the extracellular space. Defects in these functions may lead to neurodegeneration [53,56,61,73]. Thus, when astroglia are in a compromised state, this may secondarily impact the neuronal population and thus eventually lead to neurodegeneration and/or loss of neuronal functions. Aluminum pretreatment has been shown to impair the ability of astrocytes to protect neurons from glutamate toxicity [62], but the mechanism of action was only speculative. Smale et al. [69] have also demonstrated direct evidence for astrocytes undergoing an active process of apoptosis in AD brain in a postmortem study. In this vein, there is accumulated evidence for astrocytes to have a role in a number of neurodegenerative disorders of which AD is the most prevalent [1,7,8,33,48,71]. In spite of the implications of aluminum in AD, apoptotic effect of aluminum on primary culture of astrocyte is not well documented [75].

Moreover, the form by which aluminum enters brain cells as well as the intracellular consequences of aluminum in relation to neurodegenerative diseases remains unresolved [6]. Although several aluminum compounds/complexes—some of which neither exist in biological system nor consumed as such—have been studied, there is paucity of data on aluminum amino acid complexes. Aluminum glycinate is a constituent of important drugs including antacids and analgesics. Previous investigations have revealed that when volunteers took drugs containing aluminum glycinate, percentage aluminum absorbed from the intestinal tracts was 0.38%, which is more than 100-fold compared to when drugs containing $Al(OH)_3$ were taken, which was only 0.003% [44,46]. Although this may not be directly related to aluminum uptake by brain cells, however, it deserves attention. Furthermore, De Voto and Yokel [22] included some amino acids in the list of some components in serum recognized to be available to bind aluminum and glycine has the highest binding capacity than any other components in the list. Food is the primary common source of aluminum, and it is well established that amino acid concentrations of the brain extracellular fluids are affected by the quantity and composition of the food ingested [14,15,19] as well as by central nervous system (CNS) illness [43,49]. Detailed information on the uptake of aluminum amino acid complex by primary cultured astrocyte is important, therefore, for understanding the mechanism of aluminum toxicity in brain. Hence, the present study was conducted to investigate the availability of aluminum solubilized by some amino acids, especially glycine, for uptake by astrocytes. Aluminum citrate was also employed for comparison. The apoptotic effect of such internalized aluminum also forms part of the subject of the present report.

Glutamate–glutamine metabolism is central in glial metabolic activity. Methionine sulfoximine (MSO) is an irreversible inhibitor of astrocytic enzyme glutamine synthetase, and it is also known to block glutathione synthesis by inhibiting γ-glutamylcysteine synthetase [60,64,65]. Differential influence of MSO application on astrocyte transport of some amino acids has been reported [3,60]. We have therefore employed MSO to determine how the perturbation of astroglial metabolism will influence the uptake of aluminum complexed with different amino acids. Moreover, the ability of glial cells for transporting amino acids has been well established. But little is known of whether amino acid transport is, in any way, influential to the uptake and/or toxicity of aluminum in complex with it. We have therefore employed glycine and glutamate transporters blockers in the uptake studies. Ouabain, a widely known inhibitor of Na^+/K^+-ATPase, was employed to investigate whether the transport of aluminum requires energy in this form.

Thus, we describe herein the differential and real uptake of aluminum complexed with different amino acids by primary cultured astrocytes and suggested the possible mechanism. We also report how metabolic perturbation can aggravate aluminum internalization as well as the apoptotic effect of aluminum in complex with amino acid on the cells, and discussed the implications of compromised astrocytes on neurodegeneration.

2. Materials and methods

2.1. Astrocyte culture

Primary astrocyte cultures were prepared from cerebral cortices of newborn ICR mice (postnatal days 5–7) by the method previously described [50], with some modifications. Briefly, dissociated neocortical cells were suspended in appropriate volume of medium and plated at about 5 ×