[Revised manuscript]

Analysis of brain regional distribution of aluminium in rats via oral and intraperitoneal administration

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SHORT TITLE: Administration route in brain regional distribution of aluminium

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Summary

In the present work, accumulation and distribution of aluminium in the rat brain following both intraperitoneal and oral administration were studied. Electrothermal atomic absorption spectrometry was used to determine aluminium concentration in different brain areas (cerebellum, ventral midbrain, cortex, hippocampus, and striatum). The most of the brain areas showed accumulation of aluminium, but a greater and more significant increase was noted in the group receiving aluminium via intraperitoneal administration. Aluminium distribution was also dependent on the administration route used.

Keywords: Aluminium; brain distribution; intraperitoneal; oral; rat

Introduction

Aluminium is one of the most abundant metals in the earth's crust and all around present in our domestic environment. Although, it is considered a non-essential element for living organisms, the medical interest in this metal comes from the reported neurotoxicity of aluminium [1]. The bioavailability of aluminium, its ability to cross the blood-brain barrier, and the relatively slow rate of elimination from the brain contributes to guarantee the accumulation of aluminium into the brain [2], which represents an enhanced neurotoxicological risk. Thus, aluminium has been implicated in aging-related changes [3] and several neurodegenerative diseases [4]. Although, it is generally accepted that the neurotoxicity of aluminium is caused by its ability to

increase oxidative damage in the brain [5], the molecular mechanisms by which it causes neuronal damage are not fully understood [6]. Evidently, the non-redox nature of this metal, so as the extensive range of values reported in the literature concerning its accumulation in the brain, contribute greatly to this uncertain situation. The purpose of this study was to elucidate the precise localization of the absorbed aluminium in the brain following the use of two different administration routes, oral and intraperitoneal, in order to clarify its distribution in the brain and in this way contribute to understand all the factors involved in its neurotoxicity.

Materials and methods

Forty male Sprague-Dawley rats (200-250 g) were used in this study. All experiments were performed in accordance with the NIH publication "Principles of laboratory animal care" and approved by the Ethics Committee of the University of Santiago de Compostela. Animals were randomly divided into four experimental groups: the first group was daily i.p. injected with aluminium chloride (Sigma Chemical Co.) in saline (0.9 % NaCl) at a dose of 10 mg aluminium/kg/day (pH 4) for one week. The second group was i.p. injected with saline over the same period. The third group was given orally 25 mg aluminium/kg/day and 89 mg citric acid/kg/day in saline for one week. After this period, aluminium and citric acid doses were increased to 50 mg/kg/day and 178 mg/kg/day, respectively, for one more week. Finally, doses were adjusted to 100 mg aluminium/kg/day and 356 mg citric acid/kg/day for two additional weeks. Citric acid was added to enhance the gastrointestinal absorption of aluminium [15]. The fourth group was given saline orally during the entire experimental period of four weeks. Aluminium dosage was adjusted according to the animal's body weight just

before experiment. All rats received a standard diet (A04, Panlab, Barcelona, Spain) and the corresponding drinking (saline or aluminium salt+citric acid) *ad libitum*. Body weight and fluid intake were measured three times a week to adjust the doses in order to achieve a constant aluminium and citric acid intake.

After treatment, animals were sacrificed by decapitation and brains quickly excised. Regional brain segments [cerebellum (CE), ventral midbrain (VM), cortex (CO), hippocampus (H), striatum (ST)] were rapidly dissected out on ice plate, according to Paxinos and Watson [7]. Weighed brain regions were stored at -40 °C until trace element analysis. The samples were homogenised and digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), using microwave energy. A portion of 20 µL of the digested sample was introduced into the graphite furnace tube for its analysis by Electrothermal Atomic Absorption Spectrometry (ETAAS). Measurements were performed using an atomic absorption spectrometer Model 1100 B (Perkin-Elmer, Norwalk, CT, USA), equipped with an HGA-700 graphite furnace atomizer and AS-70 autosampler. A hollow cathode lamp operating at 25 mA, which provided a 309 nm line with a spectral bandwidth of 0.7 nm, was used. Deuterium background correction and pyrolytic graphite coated tubes with L´vov platforms were employed. All measurements made during this study used integrated absorbance with an integration time of 5 s. The mineralization and atomization used were 1,500 and 2,800 ºC, respectively. The volume injected was 20 µL. Each sample was digested by triplicate and measured by duplicate. Aluminium concentrations in samples were calculated using a standard addition method in an analytical range of 0-20 µg/L. The limit of detection was 0.12 µg/g. The accuracy of the method was investigated using the Reference Material DORM-2 with a certificated aluminium concentration of 10.9±1.7 µg/g, being the aluminium concentration obtained 11.4±2.2 µg/g. Reagents for aluminium determination were of

the highest available purity in order to avoid any risk of sample contamination. All glassware was washed and kept in 10 % (v/v) HNO_3 for at least 48 hours and then rinsed with ultrapure water (Milli-RiOs/Q-A10).

Results were expressed as the mean±SD. Statistical differences were tested using one-way ANOVA followed by Bonferroni's test for multiple comparisons. The accepted level of statistical significance was $p < 0.05$.

Results

As can be seen in Fig. 1, aluminium content in brain tissue was significantly increased in both aluminium-exposed groups (i.p. and oral) when compared to the corresponding control, except in VM of the i.p. aluminium-treated group.

All brain regions of oral aluminium-treated animals showed significant increased levels of aluminium in relation to their controls: +69 % CE, +200 % VM, +116 % CO, +66 % H, +143 % ST, with highest levels in the ventral midbrain>striatum>cortex, cerebellum and hippocampus. In orally-treated control animals, all areas showed a similar accumulation of aluminium.

In i.p. aluminium-treated animals, aluminium content was significantly increased compared to the respective i.p. controls (+727 % CE, +667 % CO, +877 % H and +294 % ST). Notwithstanding, the level of aluminium in VM was significantly reduced compared to that of the control group (-83 %). Aluminium in i.p. metal-treated animals seemed to accumulate preferentially in the following cerebral areas: hippocampus>cortex>striatum>cerebellum>ventral midbrain.

When comparing the two different methods of aluminium administration, we noted a greater and significant increase of aluminium in the i.p. aluminium-treated groups

compared to the orally-treated group: +77 % H, +73 % CO, +51 % CE and +39 % ST. In turn, aluminium concentration in VM was significantly reduced (-1963 %).

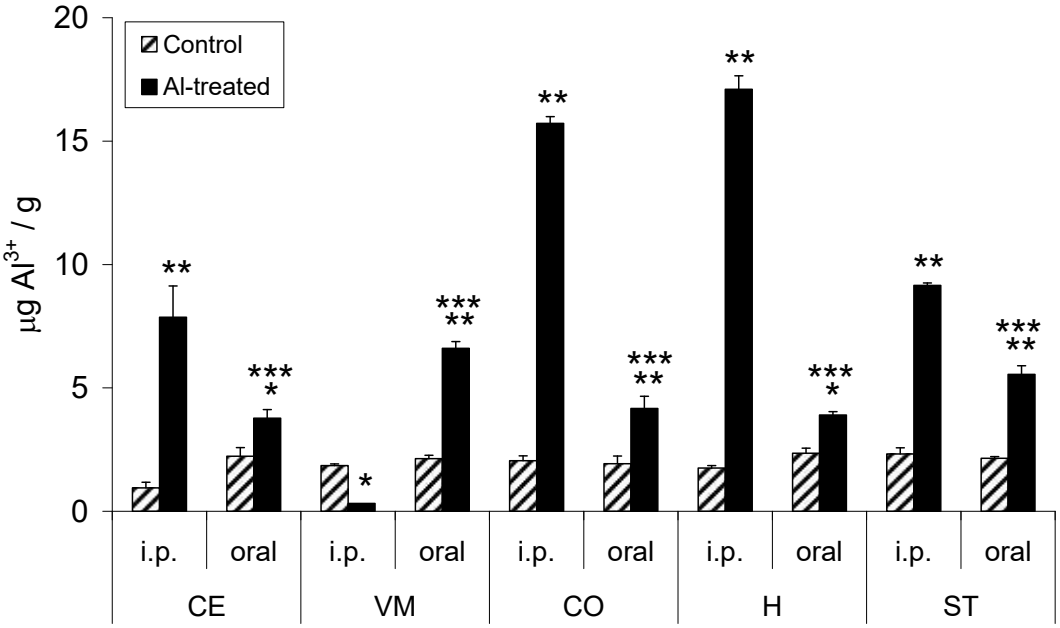

Fig. 1 Levels of aluminium in the different areas of the rat brain. Data are expressed as mean±SD. Asterisks denote values significantly different (one-way ANOVA followed by a Bonferroni's test) for treatment versus control group (*P<0.01, **P<0.001) and for the oral-treated group versus intraperitoneally-treated group (***P<0.001).

Discussion

Aluminium enters the brain through the blood-brain barrier. Previous studies suggest that there are carrier-mediated mechanisms that allow uptake of aluminium into the brain and efflux into blood [8]. It was also proposed that aluminium distribution depends on the animal species in question and the chemical form of aluminium administered [9]. The aim of the present work was to evaluate the brain regional

accumulation of aluminium in rats following the administration of aluminium chloride through two distinct administration routes: intraperitoneal and oral.

Oral administration of aluminium chloride for four weeks resulted in a significant increase in brain aluminium concentration in all the investigated brain areas. These results agree with previous studies reporting an increase of aluminium concentration in both whole brain [10] and specific areas of the brain [11, 12], which contribute to explain the impairment in motor coordination observed by some authors [13]. However, it has also been reported no significant accumulation of aluminium in the whole brain [14, 15] and even a paradoxical reduction of aluminium concentration in the whole brain of mice chronically treated with a diet containing aluminium [16]. This wide variety of results could be related with the aluminium salt used and/or the extent of brain area selected.

Our results show that i.p. aluminium chloride exposure for one week also caused a significant and greater aluminium accumulation in cerebellum, cortex, hippocampus and striatum, a finding that is consistent with previous literature concerning particular brain areas [17-20]. However, most of these publications used extensive cerebral regions. It has been also reported a no significant aluminium accumulation following i.p. administration of aluminium chloride [5]. The observation that i.p. administration of aluminium predominantly accumulates in the hippocampus is in accordance with a previous work [20]. Our present study make the interesting point that, while oral administration of aluminium chloride resulted in a significant increase of the metal concentration in the ventral midbrain, i.p. administration led to a decrease in aluminium concentration in this cerebral region. In our opinion, this curious observation could be related to the specific composition of this area and the reported ability of aluminium to

change the permeability of plasma membranes in function of their particular composition [21].

Our results clearly show that aluminium accumulation in the brain not only varied with the administration route used but also in its distribution in the distinct brain areas. Evidently, both the presence of citric acid in the oral dose of aluminium and the difference in the time of treatment also could contribute to the reported differences. However, in view of the reported variance in aluminium distribution in different cerebral regions following both treatments, it can be argued that the effects of aluminium cannot be generalized to the whole brain and a study of the kinetics of aluminium distribution in specific cerebral areas is necessary to understand the neurotoxicity of this metal and its contribution to a particular neurological disorder.

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