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Memory deficit in mice administered aluminum–maltolate complex

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Abstract

Recently, aluminum (Al) has been identified as one of the environmental factors responsible for cause certain nerve degeneration diseases, particularly, Alzheimer's disease (AD). However, the relationship between Al and AD is controversial. We previously examined whether Al induced neurotoxin in the brain of mice when aluminum–maltolate complex (ALM) was administered daily for 120 days. Our results revealed that Al accumulated in the brain induced oxidative stress, and the nerve degeneration was detected in the brain of the ALM-treated group. On the basis of these results, we have tried to examine whether the incorporated Al affects memory in mice with regard to an indicator of spatial memory deficits depending on the chemical forms of Al, namely, as an ion ($AlCl_3$) and in the form of a complex (ALM). We administered saline, $AlCl_3$, and ALM at a concentration of 40 μmol Al/kg body weight to mice by daily *ip* injections for 60 days. We assessed spatial memory by a water maze task and determined the Al levels in the brain of the mice by the neutron activation analysis method. Spatial memory deficit as an indicator of the swimming time was related to Al accumulation in the brain of mice; the chemical form of the Al compound was important in order to exhibit the memory deficit in mice; the uptake of Al is higher in mice when it is administered in a complex form than in an ionic form.

Introduction

It is well known that the ageing process is responsible for the development of certain diseases. Particularly, dementia, including Alzheimer's disease (AD), is one of the most serious diseases because it results in the loss of the individual's personality and sociality.

The causes of AD, which is characterized by senile plaques and neurofibrillary tangles (NFT), are classified into environmental and inheritance factors. Aluminum (Al) in drinking water has been proposed to be one of the environmental factors, as reported by epidemiological studies conducted since 1986 (Martyn *et al.* 1989; Forbes *et al.* 1991; Neri & Hewitt 1991; McLachlan *et al.* 1996; Gauthier *et al.* 2000). In fact, Al has been detected in both senile plaques (Candy *et al.* 1986) and NFT (Good *et al.* 1992) in the brains of AD patients. These observations were supported by the formation of NFT-bearing neurons when Al was administered in the brain of experimental animals (Klatzo *et al.* 1965). To date, several researchers have studied the relationship between Al in the brain and AD (Exley 2001); however, the pathogenic mechanism of AD with regard to the influence of Al has not yet been clarified.

Al is the third most abundant element, being twice as abundant as iron (Martin 1994). Since the distribution of Al is widespread in the biosphere, we unconsciously take up Al from food, water, air, and medicines in our day to day life. From a

quantitative point of view, over-the-counter antacids, in particular, are the most important source of Al intake in humans (Reinke *et al.* 2003). According to the WHO, the average Al intake in a day is reported to be 2.5–13 mg (WHO 1997). In addition, the WHO and FAO have suggested that the acceptable daily intake of Al is within 1 mg/kg body weight (JECFA 1989). However, this value does not necessarily indicate a safe value because of a difference in the environment and eating habits in a particular area.

In 2001 (FDA 2001), the final rule with regard to the addition of certain labeling requirements indicating the Al content in total parenteral nutrition (TPN), for specifying an upper limit of Al permitted in large volume parenterals, and requiring the applicants to submit validated assay methods to the FDA for determining the Al content in parenteral drug products, was amended in the FDA's regulations. In addition, based on the Pharmaceuticals and Medical Devices Safety Information No.179 in 2002, the Ministry of Health, Labour and Welfare, Japan stated that certain Al-containing drugs should not be used in patients undergoing renal dialysis and should not be administered continuously for a long term; in the case of patients with chronic renal failure, the doctor's or pharmacist's advice would be necessary.

Previously, we have reported (Hino *et al.* 1996; Kaneko *et al.* 2004) that the accumulation of Al is greater in the brain, liver, kidney, and spleen of mice treated with aluminum–maltolate complex ($Al(ma)_3$:ALM), which was administered daily for 120 days, than that in the untreated control mice and the $AlCl_3$-treated mice. In addition, oxidative stress was induced in the brain depending on the level of accumulated Al. On the basis of these results, we tried to examine whether the incorporated Al affects memory in mice with regard to an indicator of spatial memory deficits depending on the chemical forms of Al, namely, as an ion ($AlCl_3$) and in the form of a complex (ALM). AD is known to cause dementia, which is characterized by impairment in the understanding of time and place in patients. If Al accumulation in the brain induces spatial memory impairment, it is conceivable that Al is one of the important factors impairing brain function. Therefore, by using a water maze task, we examined the relationship between the memory of mice that were administered ALM *ip* for 60 days and Al accumulation in the brain.

Materials and methods

Materials

Aluminum chloride ($AlCl_3$) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Aluminum nitrate nonahydrate and maltol were purchased from Wako Pure Chemical Industries (Osaka, Japan). All the other reagents used were of an analytical reagent grade. Millipore water ($\sim$5 MΩ cm) that was used during the experiments was obtained by ultra-filtration of distilled water through a Milli-Q purification system (Nihon Millipore K.K., Tokyo, Japan). ALM was prepared as reported previously (Finnegan *et al.* 1987), and the physicochemical properties were checked by performing an elemental analysis and using UV spectra.

Animals

Male ddY mice aged 6 weeks (weight 37–46 g) at the initiation of the experiment were purchased from Shimizu Experimental Materials Co. (Kyoto, Japan). Their body weight was recorded at about 10 AM every day during the experiment. All experiments were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University (KPU) and were performed in accordance with the Guidelines for Animal Experimentation of KPU.

Treatment of mice

The mice were divided into 3 groups – the control group ($n = 3$), the $AlCl_3$-treated group ($n = 8$), and the ALM-treated group ($n = 8$). The control group, the $AlCl_3$-treated , and the ALM-treated groups were administered daily *ip* injections of saline, $AlCl_3$ dissolved in saline at a concentration of 40 μmol Al/kg body weight, and ALM dissolved in saline at a concentration of 40 μmol Al/kg body weight, respectively, for 60 days. All mice were given free access to drinking water and standard solid food (MF, Oriental Yeast Co., Tokyo, Japan).

Spatial memory experiment

A can (diameter: 6 cm, height: 12 cm), which served as the goal, was installed in a glass tank

(width: 60 cm, length: 30 cm, height: 35 cm). Water was poured in the tank and the goal was set at a height of 1 cm above the water surface.

Before beginning the experiment, the mice were trained for 15 days to memorize the goal. After a mouse was put on the goal for 1 min, it was transferred to a corner of the tank. Subsequently, the head of the mouse in the water was made to face a wall in a position that was opposite to the goal. The time taken by the mouse to reach the goal by swimming and to climb the goal was measured. Whenever the mouse swam to a wall of the tank before reaching the goal, the number of days was recorded. This exercise was repeated five times in a day for each mouse.

Determination of the level of Al in the brain of mice

All the mice were sacrificed under ether anesthesia and their brains were removed immediately, weighed, and lyophilized. The concentration of Al in the brain was determined by the neutron activation analysis method (NAA) at the Research Reactor Institute of Kyoto University, as reported previously (Kaneko *et al.* 2004). When the biological samples are irradiated with a neutron flux, both ^{27}Al and ^{31}P can be counted to ^{28}Al by the reactions, ^{27}Al(n, γ)^{28}Al and ^{31}P(n, α)^{28}Al, respectively. The Al levels in the brain were determined by subtracting the radioactivity that is caused by ^{28}Al originating in ^{31}P from the total radioactivity of the samples. The P concentration in the sample was determined by the malachite green assay (Kaneko *et al.* 2004).

Statistical analysis

Data are expressed as the mean ± SD and were analyzed by a Student's unpaired two-tailed *t*-test. Correlations between the parameters were tested by linear regression analysis, being tested by Pearson correlation calculations, using the GraphPad Prism software (San Diego, California, USA).

Results

Each mouse was trained for 15 days to recognize the position of the goal in the tank. Before the training, when they faced the wall, opposite the goal, and were allowed to swim, they took 10–40 s to reach the goal. However, following training, the time taken to reach the goal was shortened by approximately 2 s. The training was stopped on the 15th day when all the mice were trained to reach the goal in approximately the same amount time. After the 16th day, they received daily *ip* injections of saline or Al compounds for 60 days.

Body weight changes of mice administered saline or Al compounds for 60 days are shown in Figure 1. The control group gained body weight constantly for 30 days, following which, there was no notable change in their body weight. The $AlCl_3$-treated group gained weight gradually during the treatment period; however, their body weight was significantly suppressed as compared to that of the control group from the 30th to 45th day. The body weight of the ALM-treated group did not increase during the experiment for 60 days; this was statistically significant as compared with that of the control group after the 30th day.

The number of days at which the mice rushed to the wall before reaching the goal and that at which they took more than 2.5 s to reach the goal, as indexes for memory impairment, are summarized in Table 1. After training for 15 days, none of the mice took more than 2.5 s to reach the goal. The number of days of rushing to the wall in both the Al-treated groups significantly ($p < 0.01$) increased when compared with that of the control group. Particularly, this frequency in the ALM-

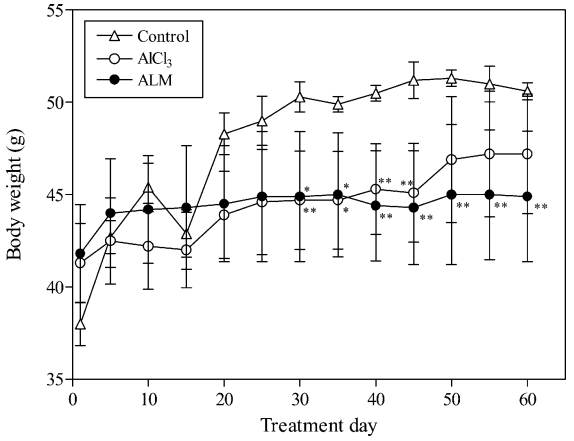

Figure 1. Body weight change in mice. The control group, $AlCl_3$-, and ALM-treated groups were given saline, $AlCl_3$ (40 μmol Al/kg body weight), and ALM (40 μmol Al/kg body weight) by *ip* injection, respectively. Each point represents the mean ± SD (the control group: $n = 3$, each Al-treated group: $n = 8$). Significance: $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* the control group.

Table 1. The number of days at which the mice rushed to the wall before reaching the goal and that at which they took more than 2.5 s to reach the goal

Treatment group	Number of mice	Number of days	
		Rushed to wall	Took more 2.5 s
Control	3	0.7 ± 0.6	0
$AlCl_3$	8	$3.9 \pm 1.6^{*}$	6.4 ± 6.4
ALM	8	$9.1 \pm 4.1^{*,**}$	15.9 ± 16.8

Data are expressed as mean ± SD. Significance: $^{*}p < 0.01$ *versus* the control group, $^{**}p < 0.01$ *versus* the $AlCl_3$-treated group.

treated group was significantly ($p < 0.01$) increased to 2.3-fold as compared with that in the $AlCl_3$-treated group. Similarly, the number of days took more than 2.5 s to reach the goal in the ALM-treated group increased to 2.5-fold as compared with that in the $AlCl_3$-treated group.

Since the effects on the memory of mice depended on the chemical form of Al administered, Al concentrations in the brain of mice that were administered $AlCl_3$ and ALM were compared (Figure 2). Al concentration in the ALM-treated group increased to 1.3-fold when compared with that in the $AlCl_3$-treated group; however, this change was not significant.

The relationship between Al accumulation in the brain of mice and the frequency of rushing to the wall or that took more than 2.5 s to reach the goal was examined. As shown in Figure 3a, the

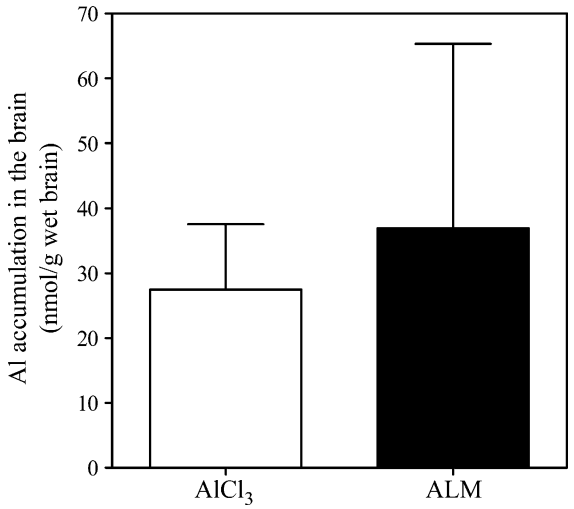

Figure 2. Al accumulation in the brain of mice given $AlCl_3$ and ALM by *ip* injections for 60 days.

regression lines were obtained as follows: $y = -0.07x + 5.87$ ($r = -0.471$) for $AlCl_3$ and $y = 0.12x + 4.49$ ($r = 0.789$) for ALM. The 95% confidence intervals (CI) of r for $AlCl_3$ were from −0.883 to 0.350, which was not significant ($p = 0.24$). In contrast, the 95% CI of r for ALM were from 0.190 to 0.960, which was significant ($p = 0.02$). In Figure 3b, the regression lines were obtained as follows: $y = -0.25x + 13.19$ ($r = -0.390$) for $AlCl_3$ and $y = 0.44x - 0.43$ ($r = 0.750$) for ALM. The 95% CI of r for $AlCl_3$ were from –0.859 to 0.434, which was not significant ($p = 0.34$); the 95% CI of r for ALM were from 0.090 to 0.951, which was significant ($p = 0.03$).

Discussion

Some metals, classified as environmental factors, have been known to cause certain nerve degeneration diseases (Montogomery 1995; Yoshida & Yoshimasu 1996; Waggoner *et al.* 1999). Of these, Al has been considered to be one of the most important factors (Kawahara 1999) contributing to the development of dialysis dementia (Alfrey *et al.* 1976), Parkinson's disease (Good & Olanow 1992), amyotrophic lateral sclerosis (Yasui *et al.* 1991), and AD (Hollosi *et al.* 1994). Based on cumulative observations indicating that a neurotoxin was expressed when Al was parenterally administered to mammals (Clayton *et al.* 1992; Lipman *et al.* 1988), and those wherein hemodialysis patients exposed to high concentrations of Al showed a decline in their memory, attention, and concentration (Bolla *et al.* 1992), Al accumulation in the brain was assumed to induce neuropathy, thereby developing the initial symptom of AD. Based on these results, we examined whether Al accumulation in the brain induces a memory deficit in animals by using a water maze task.

Before starting the experiment, the mice were trained to recognize the location of the goal in the tank. At the beginning of the training, the mice swam around and rushed to the wall of the tank. Although the mice touched the goal, they continued swimming without climbing the goal. Following the 7th day of training, the frequency of each mouse climbing the goal remarkably increased, thereby indicating that the mice memorized the location of the goal. Following the 13th day of training, we gauged that since the mice

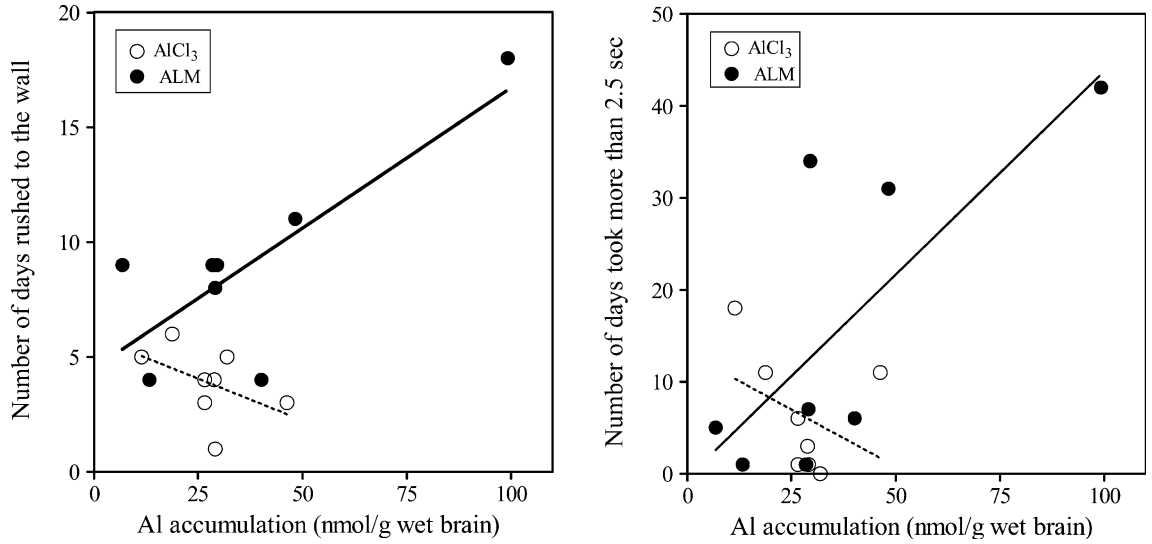

Figure 3. Relationship of Al accumulation in the brain with (a) the number of days of rushing to the wall, and (b) the number of days took more than 2.5 s to reach the goal. Dotted and solid lines show the linear regressions of the $AlCl_3$-, and ALM-treated groups, respectively. The regression lines: (a) $y=-0.07x+5.87$ ($r=0.471$) for $AlCl_3$ and $y=0.12x+4.49$ ($r=0.789$) for ALM, (b) $y=-0.25x+13.19$ ($r=0.390$) for $AlCl_3$ and $y=0.44x-0.43$ ($r=0.750$) for ALM.

completely recognized and remembered the position of the goal, they now climbed the goal, the interval from start of the experiment to reaching the goal being approximately 2 s. The training period was continued for 15 days in order to check whether the memory of each mouse was stable.

We examined the difference in the effect on spatial memory of the chemical forms of Al; $AlCl_3$ was used as an ionic form and ALM was used as a complex form. During the treatment period, the mice did not exhibit any remarkable change in behavior, regardless of the body weight of the Al-treated groups being significantly suppressed (Figure 1). The day on which the time taken to reach the goal was more than 2.5 s of the ALM-treated group was earlier than that of the $AlCl_3$-treated group, and the number of days of the ALM-treated group was more than that of the $AlCl_3$-treated group (Table 1). These results were supported by the data that Al in the brain of the ALM-treated group increases to a greater extent than that of the $AlCl_3$-treated group (Figure 2). The correlation ($r=0.750$) for the relationship between Al accumulation in the brain of the ALM-treated group and number of days took more than 2.5 s to reach the goal (Figure 3b) as well as that ($r=0.789$) between Al accumulation in the brain of the ALM-treated group and the frequency of rushing to the wall (Figure 3a) indicated the uptake of Al in the brain of mice, which in turn causes the neurotoxin to induce memory defects.

Our present results are in good agreement with the previous proposal (Miu *et al.* 2003) that long-term exposure of adult rats to Al was associated with progressive behavioral decline, including a decline in spatial memory. In addition, the present results supported a previous study (Kaneko *et al.* 2004), which demonstrated that Al accumulation and oxidative stress in the brain of the ALM-treated group were significantly higher than those of the control and the $AlCl_3$-treated groups. It is suggested that maltol enhances the bioavailability of Al (VanGinkel *et al.* 1993), resulting in its increased transport across the blood-brain barrier and retaining Al^{3+} there. This subsequently leads to a high accumulation of Al^{3+} in the brain. Furthermore, it is suggested that ALM causes death of primary cultured rat hippocampal neurons in a time- and dose-dependent manner (Kawahara *et al.* 2003) and that Neuro-2a cells treated with ALM induced apoptosis as a prominent form of cell death (Johnson *et al.* 2005).

This assumption might be supported by the fact that the control group rarely rushed to the wall during the treatment period. On the other hand,

the frequency of the ALM-treated group rushing to the wall was 13.0-fold and 2.3-fold greater than that of the control and the $AlCl_3$-treated groups, respectively (Table 1). Thus, our results may relate to important pathological changes occurring in AD that were observed in a previous study (Pratico et al. 2002). In this study, transgenic mice overexpressing a human amyloid precursor protein were fed with an Al-enriched diet, following which increased amyloid β levels and accelerated plaque deposition were observed. Additionally, they may also relate to *in vitro* experiments (Kawahara *et al.* 2001) in which chronic Al exposure induced conformational changes in the amyloid β protein by enhancing its aggregation.

In conclusion, the following findings were obtained: (1) Spatial memory deficit as an indicator of the swimming time relates to Al accumulation in the brain of the mice and (2) the chemical form of the Al compound is important in order to exhibit the memory defect in mice; the uptake of Al is higher in mice when it is administered in a complex form than when it is administered in an ionic form.

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