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Impact of Alumimum Sub-Chronic Toxicity on Body Weight and Recognition Memory of Wistar Rat

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Abstract: The aims of this study was to investigate the impact of aluminum nitrate administered in drinking water during 90 days (sub-chronic toxicity), on body weight gain, motor activity, brain aluminum accumulation and especially in recognition memory of wistar rats. Two groups of young female wistar rats were used. Treated rats received (80 mg L^{-1}) of aluminum nitrate diluted in drinking water, while control rats received a drinking water only, for 3 months. An evolution of body weight, a motor activity, object recognition memory (NOR) and brain aluminum concentration has been evaluated. The body weight was taken weekly, whereas the memory abilities and the motor activity are measured once every fortnight alternatively, by submitting rats to the open field test and to the novel object recognizing memory test. The results have showed a significant decrease in rats' body weight ($p<0.05$). Though, no significance was registered for motor activity. Nevertheless, a high significance is showed for recognition memory compared to control rats ($p<0.01$), especially at the end of testing period, even the difference between control and aluminium treated rats in brain aluminum levels was not significant.

Key words: Aluminum, sub-chronic, toxicity, rat, NOR test, open field test

INTRODUCTION

Aluminum (Al) is the 3rd abundant metallic element in the nature after oxygen and silicon; it constitutes about 8% of the Earth's crust. It is present in numerous sources, including air, food, drugs, cosmetics, vaccines, household materials and water. In Europe, 5% of total aluminum quantity ingested by human comes from drinking water and 95% comes from aliments. However, the dissolved aluminum in the water is under a particularly bioavailable shape, very easily absorbed by the digestive mucous membranes. Human exposure to aluminum is thus inevitable but neither cases of Al deficiency nor any physiological function for Al have been described as yet. For many years, Al was thought to be innocuous and largely unabsorbed from the gastrointestinal tract. Its toxicity was first recognized in 1972 and its association with a neurological syndrome in patients on prolonged hemodialysis reported. This syndrome included progressive dementia, speech difficulties, facial grimacing and motor abnormalities. Since then, further neurological syndromes have been attributed to aluminum (Arnaud and Favier, 1991; Struys-Ponsar *et al*., 1997; Terken *et al*., 2003).

In animals and in the absence of overt encephalopathy or neurohistopathology, animals exposed to soluble aluminum salts in the diet or drinking-water presented a behavioural impairment. Both rats (Commissaris *et al*., 1982; Connor *et al*., 1988) and mice (Yen-Koo, 1992) have demonstrated such impairments at doses exceeding 200 mg of aluminum per kg of body weight per day. Although, significant alterations in acquisition and retention of learned behaviour were documented (Bilkei-Gorzo, 1993; Lal *et al*., 1993).

In addition, the motor activity and body weight were also altered by aluminum intake. However, the findings in these aspects are still divergent and unclear. Colomina *et al*. (2005) and Roig *et al*. (2006) found that exposing rats to aluminum drinking water did not alter significantly the motor activity in the open field test. Nevertheless, other researcher showed that oral studies with aluminum in rats, during short-term and sub-chronic exposure, produced accumulated aluminum levels in the brain as well as altered general motor activity and impaired motor coordination (Golub *et al*., 1989; Sahin *et al*., 1995).

Concerning the body weight, Golub and Germann (2001) showed significant decreases in mice pup body weight after aluminum exposure during mother's

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gestation/lactation and offspring exposure. Nevertheless, Colomina *et al.* (2005) found that no significant alterations in body weight, food consumption, or water consumption were observed during gestation in the dams exposed to aluminum.

The aims of this study is to contribute on elucidation of the impact of aluminum nitrate administered in drinking water during 90 days (sub-chronic toxicity), on body weight gain, motor activity, brain aluminum accumulation and especially in recognition memory of wistar rats, because in our knowledge, all the studies realized in memory and investigate the spatial memory only.

MATERIALS AND METHODS

Animals and treatment: Female wistar rats, 3 months of age and 179.6±4.28 g in weight (Means±SEM, n = 14) at the beginning of the treatment, were used in this study. They were reproduced in colony room of Biology Department, Faculty of Sciences, Kenitra Morocco. The rats were housed in propylene cages under standards conditions (20°C, 50-70% humidity and 12L: 12D cycle). They were given free access to food (SNV, Temara, Morocco) and tap water. The control rats (n = 7) were given tap water and the aluminum intoxicated rats (n = 7) received 80 mg L^{-1} of aluminum nitrate (Merck). Aluminum nitrate was diluted in tap water and given to animals during 90 days.

Behavioral experiments

Open field behavior: An open field test was conducted between 09:00 and 11:00 am to examine the possible effect of sub chronic aluminum intoxication on behavior in a novel environment every fortnight from the beginning to the end of intoxication period.

Apparatus consisted of an open top wooden box (100×100×40 cm) covered by white consistent plastic. Floor area was marked into 25 squares and illuminated in the center by a 60 W halogen bulb suspended 100 cm above.

Animals were placed in the center of the open field and behavior was videotaped for 7 min for each rate. Open field behaviors were scored by a trained observer who was blind to the treatment conditions. The measures scored consisted on horizontal activity (number of squares crossed).

Novel Object Recognition (NOR) memory task: The apparatus and procedures for NOR training have been described elsewhere (Ennaceur *et al.*, 2004; De Lima *et al.*, 2005). The task took place in a 40×50 cm^2 open field surrounded by 50 cm high walls, made of plywood covered by black fine plastic layer. All animals were given a habituation session where they were left to freely exploring the open field for 5 min. No objects were placed in the box during the habituation trial. Twenty-four hours after habituation, NOR training was conducted by placing individual rats for 5 min into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. In a long-term retention test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object (A) and a novel object (B).

A single set of three objects was used for all animals. All objects presented similar textures, colours and sizes, but distinctive shapes. The index of recognition memory was defined as ratio of exploration object B number and the sum of exploration object A and B number. Between trials the objects were washed with 10% ethanol solution.

Exploration of an object was defined as directing the nose to the object at a distance≤1 cm and/or touching it with the nose; conversely, turning around or sitting on the object was not considered as exploratory behaviour. NOR procedures were conducted in a presence of luminescent source (60 w) from 1 m in the top of the apparatus.

The test took place every fortnight alternatively with the open field test.

Brain aluminum evaluation: The day after the last test, aluminum concentration was estimated in control and treated rat's brain by graphite furnace atomic absorption spectrometry (Perkin Elmer 1100) with deuterium back ground correction. Rats were anesthetized by the chloral 7% and killed by decapitation. The whole brain was extracted from the skull. Tissues samples (0.1-0.3 g) were dried, milled and digested by HNO_3 acid (4 mL) 65% (Merck). All the analyses were performed in triplicate and the results were expressed in μg g^{-1} tissue wet weight. All the vessels and their caps used were previously washed in hydrochloric acid and then in 1% nitric acid (Merck) for a week and rinsed in ultrapure water, to prevent any contamination (Pinta, 1980; Struys-Ponsar *et al.*, 1997).

Statistical analyses: Data obtained was expressed as Mean±SEM. To evaluate the differences between control and treated groups, the non parametric Mann-Whitney U-test was used. A p-value smaller than 0.05 was considered to reflect a statistically significant difference.

RESULTS

The body weight gain: There was no significant difference in body weight between control (C) and aluminium

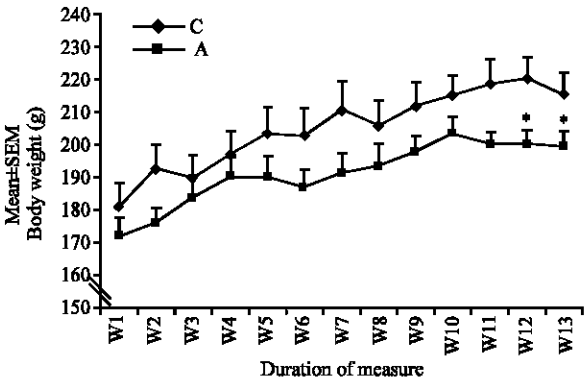

Fig. 1: The record of weight gain shown for the 13 weeks of aluminum exposure. *Statistically difference was showed in the end of test (W12 and W13, $p<0.05$)

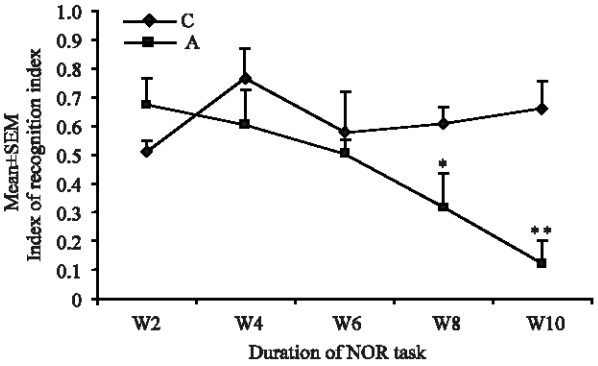

Fig. 2: Mean of recognition memory index (ratio of object B exploration number and the sum of object A and B exploration number) of every week respecting a fortnight period between recognition memory and open field test. *, **A higher difference between control (C) and treated groups (A) was registered at week 8 ($p<0.05$) and week 10 ($p<0.01$) of recognition memory test

treated rats (A) in the first eleven weeks. However, the significance was showed at the end of testing period (W12 and W13, $p<0.05$) (Fig. 1).

Motor activity in open field test (OF): The mean crossed squares exploring by both control (C) and treated rat by aluminum (A) in (OF) test was not significantly different ($p>0.05$) in each fortnight testing and during the Whole period (90 days) (Table 1).

Index of recognition memory: The effect of aluminium on rat recognition memory, during the whole period, was significant ($p<0.05$). The mean of the index of recognition memory was 0.63±0.04 in control (C), against 0.45±0.06 in the aluminium treated rats (A) (Table 1).

Table 1: Mean of squares crossed number (SC number), of recognition memory index and of aluminum brain levels, during 90 days in control rats (C) and in aluminum treated rats

Parameters	C group	A group
SC number during 90 days	38.3±4.52[a]	40.9±5.03[a]
Index of recognition memory during 90 days	0.63±0.04[a]	0.45±0.06[b]
Brain aluminum levels	0.83±0.14[a]	1.2±0.19[a]

Values with different superscript letter(s), for each parameter, are significantly different ($p<0.05$)

The study of this effect in each fortnight showed that the significant effect appears at week 8 ($p<0.05$) and week 10 ($p<0.01$) (Fig. 2).

Brain aluminum evaluation: The aluminium concentration didn't show any signification between both groups $p>0.05$ (Table 1).

DISCUSSION

The current findings showed that aluminum affects significantly the rats' body weight at the end of the experiment (after 90 days exposure). This result is consistent with others researches which found that exposure of different aluminum salts decreased rats' and hamsters' body weight (Drew *et al*., 1974; Stone *et al*., 1979). In addition, Golub and Germann (2001) showed significant decreases in mice pup body weight after aluminum exposure during mother's gestation/lactation and offspring exposure. This decrease found accompanied by reduction in water consumption of rats (Hicks *et al*., 1987).

In this study, the body weight' decrease may be explained by a possible aluminum effect on brain and kidneys which control drinking behaviour. Actually, many studies have shown that aluminum is found to accumulate in these organs (Alfrey *et al*., 1980; Greger *et al*., 1986; Domingo, 1987). Otherwise, the decrease of serum triglycerides and mitochondrial energy metabolism after different rats aluminum salts exposure, can be another hypothesis (Panda *et al*., 2008; Sugawara *et al*., 1988).

In this study, the aluminum exposure didn't show any significant effect on motor activity in open field test either in short-term and sub-chronic aluminum exposure. These results are in accordance with other studies that found no alterations in open field behaviour (Connor *et al*., 1988; Jope and Johnson, 1992; Domingo *et al*., 1996; Colomina *et al*., 2005).

With respect to the effect on memory, this study demonstrates that recognition memory was affected by this intoxication. This impact was clearly proved in the end of the testing period, suggesting that the administered dose is weak to induce a quick effect. This

suggestion is supported by the brain aluminum content which showed no significant difference between control and intoxicated rats.

In the other researches, it is reported that aluminum decreased the rats' maze-learning ability after 90 days of aluminum salts treatment (Bilkei-Gorzo, 1993). In addition, Lipman *et al.* (1988) showed that memory impairment, particularly for short term memory, is characteristic of the aluminum-associated encephalopathies including Alzheimer's disease, suggesting a deficit in memory acquisition (learning) and consolidation.

The findings results concerning the impairment in recognition memory may be explained by the aluminum effect on hippocampus functioning and on long-term potentialisation impairments (Platt *et al.*, 1995; Gilbert and Shafer, 1996).

Furthermore, a long retention interval studies have shown that hippocampus is implicated in object recognition memory. In that report, it was observed a delay-dependant hippocampal involvement (Vnek and Rothblat, 1996; Clark *et al.*, 2000; Hammond *et al.*, 2004).

CONCLUSION

In conclusion, this study demonstrates that the aluminum sub-chronic toxicity, at the used concentration, didn't affect the motor activity and brain aluminum amounts, but it decreases body weight and recognition memory faculties. Though, the process is still ambiguous and requires more detailed studies.

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