EFFECTS OF PRENATAL ALUMINUM TREATMENT ON DEVELOPMENT AND BEHAVIOR IN THE RAT

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ABSTRACT—Developmental and behavioral effects of aluminum administration were studied in THA rats. To three groups of pregnant rats were administered single dose of 0, 900 or 1,800 mg/kg of aluminum chloride on the day 15 of gestation by gavage.

Significant differences were observed between the aluminum treated offspring and controls in terms of body weight, timing of pinna detachment and eye opening, and appearances of auditory startle. Slower learning acquisition was observed in treated groups. The longer latency and more rearings in the open field test were observed in 1,800 mg/kg treated females.

These results suggest that single dose of aluminum chloride during prenatal period affects the development and behavior in rats.

Key words : aluminum chloride, toxicity, rat, THA strain, single dose, gavage, prenatal administration, neuromotor maturation, Sidman avoidance test, open field test.

INTRODUCTION

Aluminum, which is the most abundant metal in environment, is contained in numerous foods and drugs, water and other sources. It is known as neurotoxic agent (Boegman and Bates, 1984; Krishnan et al., 1988), and has been implicated as a possible agent in dialysis dementia (Wills and Savory, 1985).

Although many studies are reported on the toxic effects of aluminum (Conner et al., 1988; Klein et al., 1988; Sjögren et al., 1985; Yokel and McNamara, 1989), few studies have been reported on the developmental (Wisniewski et al., 1980; Golub et al., 1992) and behavioral effects (Petit et al., 1985; Thorne et al., 1987; Yokel, 1989) of aluminum.

The purpose of present study is to evaluate

the effects of single dose of aluminum treatment during prenatal period on development, behavior and learning in rats.

MATERIALS AND METHODS

Animals and treatment : Female rats of the 22nd generation of THA strain (Shigeta et al., 1989; Shigeta et al., 1990), which has high and stable learning abilities, were mated with same-aged males. The day when vaginal plug was present was designated as day 1 of gestation. Ten pregnant rats were individually housed in a plastic cage and bred in a clean-air system (Clean Rack CL-5413; CLEA Japan Inc.). This system was placed in the breeding room controlled at the temperature of $23 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$ and with alternating 12 hr light cycle (light periods, 08:00–20:00). Foods (CE-2, CLEA Japan Inc.) and tap water was given *ad libitum*.

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On day 15 of gestation, pregnant rats were divided into three experimental groups randomly. The first group (Group I) with four rats, the second group (Group II) with three rats, and a control group with three rats. Aluminum chloride (purity: 98%, Wako Chemical Co., Japan) dissolved in saline was administered at a dose of 900 mg/kg and 1,800 mg/kg by gavage, respectively for Group I and II rats. The control group received only saline by gavage. These solutions were prepared to give a dose in a volume of 1 ml/kg body weight based on the day 1 of gestation. Doses of aluminum chloride in this experiment correspond approximately with 1/4 and 1/2 of the acute oral LD₅₀ of aluminum chloride for adult rats.

The day of parturition was designated as postnatal day 0. Litters were culled to a maximum size of eight with balanced for sex and assigned to dam on the postnatal day 1. In this study, we used all of these pups. Twenty offspring from three dams for control group (10 males and 10 females), twenty-eight offspring from four dams for Group I (10 males and 18 females) and eight offspring from one dam for Group II (2 males and 6 females) were provided to the experiment. Pups were weaned at postnatal day 21. Males or females were housed in cages separately in groups to avoid the stress and accompanying disruption of behavior caused by isolation housing. The rats were bred in the same environmental condition as their dams and freely given food and water.

Developmental indices: Body weights of litters were recorded on postnatal days 1, 7, 14, 21 and 28. The initiations of pinna detachment, incisor eruption and eye opening were observed. Surface righting (Vorhees et al., 1979), cliff avoidance (Vorhees et al., 1979), pivoting (Vorhees et al., 1979) and auditory startle (Miyake et al., 1989) were tested as indices for neuromotor and sensory maturation of litters.

Behavioral tests: Sidman avoidance test to assess the learning ability of offspring was performed from 4 weeks of age. The system of the operant behavioral test consisted of Skinner boxes, a teletypewriter, and a behavioral-controlling apparatus (BRS/LVE Co., USA). This system was operated under the control of a minicomputer. The schedule of Sidman avoid-

dance test was as follows: shock-shock interval 5 sec, response-shock interval 30 sec, shock intensity DC100V, 3 mA, 0.5 sec. All avoidance tests were performed for 60 min a day for 10 days. The avoidance data was collected and analyzed the first 30 min of one-hour test for acquisition speed. The data from the later 30 min of test was used to evaluate the acquisition level. All data were expressed as avoidance rates: $Ar = (Ts - Rs) / Ts \times 100$, Ar: Avoidance rate (percentage), Ts: Total number of electric shocks when no responses occurred, Rs: Total number of shocks given to the rat.

Open field test to assess the emotionality was performed with the offspring at 10 weeks of age. Open field activity of the rat was recorded in a 100^W × 100^D × 40^H cm open black acrylic box. The floor of box was divided into 16 squares, and a 25 × 25 cm starting area was made on the center of floor by white painted lines. Latency to exit from starting area, ambulation, rearings, defecation and urination were recorded for 3 min in this test.

Statistical analysis: Data summaries and statistical analysis were prepared with statistical software (SAS version 6.03). Growth, neurobehavioral parameters and activity measures were evaluated with ANOVA and the Scheffe's multiple comparison test. When P values were equal to and/or less than 0.05, they were defined to be significant difference.

RESULTS

Dam data: Two of three dams in Group II died immediately after administration. These dams were examined by autopsy, but no significant abnormalities were showed. Effect of aluminum treatment was not observed in the remaining dam in Group II and all dams in Group I. No significant difference in the body weight gain from the conception to parturition and other toxic signs by macroscopic examination were observed among the all survived groups. All survived dams delivered. No differences in the mean number of implantation sites, birth rate, mean litter size and maternal behaviors were observed among the three groups of rats.

Pup data: Body weights of the three groups are indicated in Table 1. The body weights of

treatment rats were commonly lower than controls from birth to 28th day and significant differences were present between control and treatment groups.

The days of initiation of the developmental signs of three groups are indicated in Tables 2 and 3. No difference was seen among three groups in the timing of the incisor eruption. Delays with dose-dependent effect in the timing of pinna detachment and eye opening were observed in the two treated groups and significant differences between controls were showed. No difference was observed among the three groups in surface righting, cliff avoidance and pivoting. The appearances of auditory startle in males of the two treated groups were significantly delayed than controls.

The avoidance rates of the three groups during the first 30 min of tests are shown in Table 4. Mean avoidance rates of males of Group II were approximately twenty percent lower than

that of other two groups from the first to second session. Lower avoidance rates, approximately ten to fifteen percent, were indicated in the females of Group II compared with control from the first to third session. Although slow learning acquisitions on the early stage of test were seen in both males and females of Group II, no statistical differences were seen among three groups. No apparent difference in the avoidance rates during the later 30 min of the tests was indicated among the three groups (data not shown).

The activities for 3 min in the open field test of the three groups are shown in Table 5. There were no differences in the start latency, ambulation, rearing, defecation and urination among the three groups of males. On the contrary, females of Group II tended to exhibit a longer latency and a few rearings than that of other two groups. Ambulation of females in Group II was significantly less than that of other two groups.

Table 1. Body weight in three groups of rats.

	Males			Females		
	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)
Number of rats	10	10	2	10	18	6
Day 1	7.5 ± 0.8	5.5 ± 0.9**	4.8 ± - **	7.1 ± 0.9	5.4 ± 1.0**	4.7 ± 0.1**
Day 7	15.8 ± 0.9	14.2 ± 1.6*	12.6 ± - *	15.4 ± 1.1	12.2 ± 1.8*	12.7 ± 0.7*
Day 14	28.9 ± 1.8	26.8 ± 1.9	25.0 ± - *	28.6 ± 1.3	24.8 ± 1.2*	24.4 ± 1.1*
Day 21	45.3 ± 4.5	40.9 ± 2.5	39.2 ± - *	45.6 ± 2.6	40.1 ± 3.2	39.2 ± 1.6*
Day 28	87.7 ± 5.7	84.0 ± 6.3	76.5 ± -	83.6 ± 3.4	81.6 ± 2.3	78.3 ± 2.4

Weight in grams.

Values indicate as mean ± S.D. except males in Group II.

*: P<0.05 and **: P<0.01 vs. control.

Table 2. Days of age at appearance the developmental sign.

	Males			Females		
	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)
Number of rats	10	10	2	10	18	6
Pinna detachment	2.0 ± 0.0	2.4 ± 0.5	3.0 ± - **	2.0 ± 0.0	2.7 ± 0.8*	3.0 ± 0.0*
Incisor eruption	10.2 ± 1.0	10.2 ± 0.4	10.5 ± -	10.0 ± 0.9	10.4 ± 0.9	10.3 ± 0.5
Eye opening	14.1 ± 0.6	14.5 ± 0.7	15.0 ± -	13.5 ± 0.5	14.7 ± 0.8**	15.0 ± 0.0**

Values are expressed as mean ± S.D. except males in Group II.

*: P<0.05 and **: P<0.01 vs. control.

Table 3. Neurobehavioral development in three groups of rats.

	Males			Females		
	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)
Number of rats	10	10	2	10	18	6
Surface righting	9.5 ± 2.4	9.6 ± 1.6	11.5 ± -	10.2 ± 2.2	11.1 ± 2.3	10.7 ± 2.2
Cliff avoidance	4.3 ± 0.8	4.4 ± 0.8	4.5 ± -	4.4 ± 0.9	4.5 ± 0.9	4.3 ± 0.5
Pivoting [†] Day 7	4.6 ± 2.0	4.8 ± 2.6	2.3 ± -	7.8 ± 7.4	5.6 ± 3.9	4.3 ± 1.7
Day 9	4.1 ± 2.7	5.3 ± 3.1	3.9 ± -	4.7 ± 2.5	6.4 ± 5.7	6.5 ± 4.1
Day 11	5.1 ± 3.5	3.7 ± 1.2	1.5 ± -	3.8 ± 1.6	5.4 ± 2.8	4.1 ± 1.9
Auditory startle	11.7 ± 1.1	12.8 ± 0.6*	13.5 ± - *	12.0 ± 0.7	12.9 ± 1.2	13.0 ± 0.6

Values are expressed as mean ± S.D. except males in Group II.

Value indicate as days or seconds (†).

*: P<0.05 vs. control.

Table 4. Avoidance rate (percentage) of three groups of rats in the first 30 min of one hour test.

	Males			Females		
	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)
Number of rats	10	10	2	10	18	6
Session (day)						
1	58.1 ± 8.7	58.9 ± 10.8	39.5 ± -	60.6 ± 10.5	60.0 ± 11.0	44.9 ± 23.2
2	80.6 ± 5.5	80.2 ± 13.6	61.2 ± -	80.1 ± 9.2	74.5 ± 14.9	65.5 ± 18.3
3	88.2 ± 4.3	83.3 ± 8.1	82.1 ± -	80.7 ± 16.6	80.0 ± 18.4	70.7 ± 25.1
4	87.2 ± 9.3	85.5 ± 11.5	86.1 ± -	87.6 ± 16.5	82.9 ± 15.6	80.9 ± 17.0
5	92.9 ± 6.9	92.4 ± 10.5	92.6 ± -	92.3 ± 5.2	93.0 ± 4.0	91.8 ± 3.6
6	93.5 ± 5.3	91.3 ± 8.6	90.7 ± -	95.4 ± 3.6	91.6 ± 11.8	91.6 ± 4.4
7	94.3 ± 6.5	90.8 ± 6.3	88.7 ± -	95.3 ± 3.9	94.2 ± 5.2	93.1 ± 4.3
8	95.8 ± 2.9	91.6 ± 6.0	92.5 ± -	94.0 ± 6.3	93.7 ± 9.3	92.3 ± 3.9
9	95.0 ± 4.9	94.8 ± 6.0	93.6 ± -	95.7 ± 2.3	94.9 ± 6.2	90.3 ± 5.3
10	95.8 ± 4.1	94.5 ± 2.9	94.3 ± -	93.5 ± 5.3	93.3 ± 8.2	92.8 ± 4.4

Values are expressed as mean ± S.D. except males in Group II.

Table 5. Activities for three minutes in open field test.

	Males			Females		
	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)	Control	900 mg/kg (Group I)	1800 mg/kg (Group II)
Number of rats	10	10	2	10	18	6
Latency (sec)	5.7 ± 3.8	4.6 ± 3.6	5.4 ± -	4.9 ± 1.9	5.6 ± 2.6	8.6 ± 4.5
Ambulation	42.4 ± 9.5	42.7 ± 7.6	43.0 ± -	58.5 ± 7.4	52.5 ± 11.7	38.3 ± 11.2*
Rearings	23.5 ± 4.8	24.9 ± 5.6	21.5 ± -	21.8 ± 7.1	21.7 ± 5.9	14.5 ± 3.4
Defecation	3.4 ± 1.0	3.1 ± 1.7	3.5 ± -	2.5 ± 1.6	2.2 ± 1.9	1.5 ± 1.3
Urination	1.8 ± 1.8	0.9 ± 0.7	1.0 ± -	0.8 ± 0.8	0.6 ± 0.6	1.2 ± 1.0

Values are expressed as mean ± S.D. except males in Group II.

*: P<0.05 vs. control and Group I.

DISCUSSION

Results of this experiment demonstrate that single dose of aluminum treatment, both low dose group and high dose group, during prenatal period can influence development and behavior in rats. Effects of aluminum treatment are expressed by the lower body weight, delay of neuromotor maturation and learning acquisition, and lower activity.

Bernuzzi et al. (1986, 1989) reported the growth retardation with dose-dependent effect and alterations in neuromotor maturation of rats (Wistar strain) delivered from dams treated aluminum chloride or lactate during pregnancy. Golub et al. (1989) indicated the motor activity in adult female Swiss-Webster mice fed 1,000 $\mu\text{g/g}$ Al in diet for six weeks was about twenty percent lower than in controls. Donald et al. (1989) reported the maternal exposure to aluminum lactate (1,000 $\mu\text{g Al/g}$ diet) during gestation and lactation caused neurobehavioral deficits in weanling Swiss-Webster mice. In this study, lower body weight and delays of pinna detachment and auditory startle with dose-dependent effects were observed, both males and females, in pups delivered from treated dams. Females in single dose of 1,800 mg/kg aluminum chloride treated group showed longer latency, a few ambulation and rearings than other females in the open field test. These results suggest that single dose of 1,800 mg/kg aluminum chloride treatment to dams suppress the activity of their pups. Findings of our study agree with the results of previous reports.

Commissaris et al. (1982) reported the impaired retention of shuttle box avoidance in rats fed 2,000 $\mu\text{g Al/g}$ diet for 5 weeks and slower learning acquisition of the same test in rats fed 1,000 $\mu\text{g Al/g}$ diet for 11 months. Muller et al. (1990) reported a orally treatment with 400 mg Al/kg/day aluminum lactate in Wistar rats during gestation has affected to neuromotor maturation and learning abilities of their pups.

Results of the present study indicated low avoidance rates during the initial stages of the test and apparent delay in learning acquisition among aluminum treated rats. These findings are correspond to results of the Commissaris et al.

(1982) and Muller et al. (1990).

Our study was designed to clarify the developmental and behavioral effects of single dose of aluminum treatment during prenatal period in rats. The results of the present investigation suggest that the effects of single dose of 900 mg/kg and 1,800 mg/kg aluminum administration on 15th day of gestation can induce long-term effects on the central nervous system.

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REFERENCES

- Bernuzzi, V., Desor, D. and Lehr, P. R. (1986) : Effects of prenatal exposure on neuromotor maturation in the rat. *Neurobehav. Toxicol. Teratol.*, **8**, 115–119.
- Bernuzzi, V., Desor, D. and Lehr, P. R. (1989) : Developmental alterations in offspring of female rats orally intoxicated by aluminum chloride or lactate during gestation. *Teratology*, **40**, 21–27.
- Boegman, R. J. and Bates, L. A. (1984) : Neurotoxicity of aluminum. *Can. J. Physiol. Pharmacol.*, **62**, 1010–1014.
- Commissaris, R. L., Cordon, J. J., Sprague, S., et al. (1982) : Behavioral changes in rats after chronic aluminum and parathyroid hormone administration. *Neurobehav. Toxicol. Teratol.*, **4**, 403–410.
- Conner, D. J., Jope, R. S. and Harrell, L. E. (1988) : Chronic oral aluminum administration to rats : Cognition and cholinergic parameters. *Pharmacol. Biochem. Behav.*, **31**, 467–474.
- Donald, J. M., Golub, M. S., Gershwin, M. E., et al. (1989) : Neurobehavioral effects in offspring of mice given excess aluminum in diet during gestation and lactation. *Neurotoxicol. Teratol.*, **11**, 345–351.
- Golub, M. S., Donald, J. M., Gershwin, M. E., et al. (1989) : Effects of aluminum ingestion on spontaneous motor activity of mice. *Neurotoxicol. Teratol.*, **11**, 231–235.

- Golub, M. S., Keen, C. L. and Gershwin, M. E., (1992): Neurodevelopmental effect of aluminum in mice: Fostering studies. *Neurotoxicol. Teratol.*, **14**, 177-182.
- Klein, G. L., Heyman, M. B., Lee, T. C., et al. (1988): Aluminum-associated hepatobiliary dysfunction in rats: Relation-ships to dosage and duration of exposure. *Pediatr. Res.*, **23**, 275-278.
- Krishnan, S. S., McLachlan, D. R., Krishnan, B., et al. (1988): Aluminum toxicity to the brain. *Sci. Total Environ.*, **71**, 59-64.
- Miyake, K., Misawa, T., Aikawa, H., et al. (1989): The effects of prenatal trimethyltin exposure on development and learning in the rat. *Jpn. J. Ind. Health*, **31**, 363-371. (in Japanese)
- Muller, G., Bernuzzi, V., Desor, D., et al. (1990): Developmental alterations in offspring of female rats orally intoxicated by aluminum lactate at different gestation periods. *Teratology*, **42**, 253-261.
- Ondreicka, R., Ginter, E. and Kortus, J. (1966): Chronic toxicity of aluminum in rats and mice and its effects on phosphorus metabolism. *Brit. J. Ind. Med.*, **23**, 305-312.
- Petit, T. L., Biederman, G. B., Jonas, P., et al. (1985): Neurobehavioral development following aluminum administration in infant rabbits. *Exp. Neurol.*, **88**, 640-651.
- Shea, T. B., Clarke, J. F., Wheelock, T. R., et al. (1989): Aluminum salts induce the accumulation of neurofilaments in perikarya of NB2a/dl neuroblastoma. *Brain Res.*, **492**, 53-64.
- Shigeta, S., Misawa, T., Yoshida, T., et al. (1989): Neurobehavioral analysis of high-rate Sidman avoidance rat strain. *Jpn. J. Psychopharmacol.*, **9**, 217-224.
- Shigeta, S., Miyake, K., Misawa, T., et al. (1990): A new inbred rat strain "THA". *Rat News Lett.*, **23**, 9-11.
- Sjögren, B., Lidums, V., Hakansson, M., et al. (1985): Exposure and urinary excretion of aluminum during welding. *Scand J. Work Environ. Health*, **11**, 39-43.
- Thorne, B. M., Donohoe, T., Lin, K-N., et al. (1986): Aluminum ingestion and behavior in the Long-Evans rat. *Physiol. Behav.*, **36**, 63-67.
- Thorne, B. M., Cook, A., Donohoe, T., et al. (1987): Aluminum toxicity and behavior in the weaning Long-Evans rat. *Bull. Psychonom. Soc.*, **25**, 129-132.
- Vorhees, C. V., Butcher, R. E., Brunner, R. L., et al. (1979): A developmental test battery for neurobehavioral toxicity in rats: a preliminary analysis using monosodium glutamate calcium carrageenan, and hydroxyurea. *Toxicol. Appl. Pharmacol.*, **50**, 267-282.
- Wills, M. R. and Savory, J. (1985): Water content of aluminum, dialysis dementia, and osteomalacia. *Environ. Health Perspect.*, **63**, 141-147.
- Wisniewski, H. W., Sturman, J. A. and Shek, J. W. (1980): Aluminum chloride induced neurofibrillary changes in the developing rabbit: A chronic animal model. *Ann. Neurol.*, **8**, 479-490.
- Yokel, R. A. (1983): Repeated systemic aluminum exposure effects on classical conditioning in the rabbit. *Neurobehav. Toxicol. Teratol.*, **5**, 41-46.
- Yokel, R. A. (1989): Aluminum produces age related behavioral toxicity in the rabbit. *Neurotoxicol. Teratol.*, **11**, 237-242.
- Yokel, R. A. and McNamara, P. J. (1989): Elevated aluminum persists in serum and tissues of rabbits after a six-hour infusion. *Toxicol. Appl. Pharmacol.*, **99**, 133-138.