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Effects of Postnatal Aluminum Lactate Exposure on Neuromotor Maturation in the Rat

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In alkaline or neutral soils, aluminum is insoluble, but its solubility progressively increases with acidity, so acid precipitations have a considerable influence in mobilizing aluminum in natural waters, leading to higher alimentary ingestion of this element. In normal subjects aluminum is absorbed by the gastrointestinal tract and is excreted in urine. But even discrete renal failure may lead to Al accumulation in various tissues. Certain neurologic deseases have been related to Al intoxication : Amyotrophic lateral sclerosis and Parkinsonism-dementia of Guam (Perl et al 1982), senile dementia of Alzheimer type (Crapper et al 1976, Krishnan et al 1987). In patients undergoing chronic hemodialysis and ingesting aluminum-containing drugs, Al exposure is considered to be the causal factor for a high incidence of "dialysis encephalopathy" (Alfrey et al 1976). Microcytic anemia and osteomalacia usually appeared before the neurologic symptoms (Parkinson et al 1981, Wills and Savory 1983). This appears to be particularly prevalent in young children with azotemia, who receive large doses of aluminum containing phosphate-binding gels (Andreoli et al 1984). We have recently reported that the surviving pups of rats treated with aluminum during gestation showed a delay in their neuromotor development, as well as weight delay during the first postnatal week (Bernuzzi et al 1986). This paper examines the effects of postnatal aluminum lactate exposure on mortality, weight evolution and neuromotor maturation in the rat.

MATERIALS AND METHODS

Eleven Wistar pregnant female rats (Iffa Credo, l'Arbresle, France) were used. The animals were housed in individual plastic cages, allowed free access to food and water, and maintained under constant environmental conditions (22-23 °C and alternate cycle of 12 hr light / darkness). Each litter was randomly divided into four groups: a control group and three groups which received 100, 200 or 300 mg Al/kg body weight / day respectively.
Each pup was tattooed with spots of alizarine blue. Aluminum lactate, dissolved in distilled water (0.3 to 0.5 ml) was

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administered by gastric intubation to newborn rats; the control pups received an equal volume of the vehicle. All pups were treated from postnatal day 5 to 14. The numbers of pups at the beginning of the experiment were respectively 25, 25, 29 and 38 in control, group 1 (100 mg Al/kg), group 2 (200 mg Al/kg) and group 3 (300 mg Al/kg). Young rats were counted and weighted every day throughout the treatment and on day 20. Each pup was subjected to different tests allowing a rapid evaluation of their neuromotor maturation level: grasping reflex (postnatal day 6: the young was placed on an initially horizontal rotating wire-netting board and had to avoid falling by grasping: angle at falling was measured and reaching vertical position was considered as success); negative geotaxis (day 9: the rat was placed with head down on an inclined plane at an angle of 20° from the horizontal position: time spent to make a 180° turn to come back with head upward was measured); suspension test (day 12: time of remaining hung to a horizontal steel thread (0.5 mm in diameter) was measured); locomotor coordination test (day 20: the rat, put in water, could reach a platform by climbing up a 30 cm high metal rod; time to put the 4 feet on the platform was measured). For more details concerning these tests, see Bernuzzi et al 1986.

Analysis of body weight data were performed using single-factor analysis of variance and Scheffe's test for multiple comparisons. Mortality and behavioural data were analysed by non-parametric statistics: X2, Kruskal-Wallis and Mann-Whitney test. Statistical analysis were performed using, for each litter, the mean of data from pups receiving a given treatment as statistical units. To avoid possible statistical bias, data from dead pups were excluded from all the evaluations.

RESULTS AND DISCUSSION

Table 1. Postnatal mortality rate in pups. (in % of initial population in each group).

	Control	Treated Groups G1	G2	G3
Day 5	0	0	0	0
Day 6	0	0	0	7.89
Day 8	0	0	0	21.05
Day 10	4	4	13.79	36.84
Day 12	4	12	24.13	47.37
Day 14	4	24	31.03	57.89
Day 20	4	24	31.03	63.15

Mortality of pups (table 1) was analysed by X2 test applied day by day to the number of dead pups in the four groups. The intoxication

Table 2. Weight evolution of pups. (Weights in g : Mean ± Standard deviation).

	Control	Treated Groups G1	G2	G3
Day 5	11.7±2	11.6±1.7	11.5±1.7	11.1±1.6
Day 7	16.0±3.1	14.5±2.8	12.5±1.9	11.4±1.5
Day 8	18.5±3.4	16.3±3.2	13.0±2.2	11.6±1.4
Day 10	23.9±3.4	19.8±4.5	15.9±2.6	12.7±2
Day 12	28.3±4.2	25.0±3.9	20.1±3.6	15.1±2.5
Day 14	34.7±4.1	31.3±4.2	24.4±3.7	17.7±3.5
Day 20	43.1±5.2	39.1±6.3	28.3±5.9	23.4±6.5

induced an increase in death rate, specially in animal receiving 300 mg Al/kg/day (group 3). A significant difference occured on the 4th day following the first treatment in group 3 pups (global $X2 = 17.85$, 3 df, $p<0.001$; comparison group 3 vs. control: $X2 = 4.28$, 1 df, $p<0.05$), and on the 10th day in group 2 pups (global $X2 = 21.84$, 3 df, $p<0.001$; comparison group 3 vs. control: $X2 = 18.90$, 1 df, $p<0.001$; comparison group 2 vs. control: $X2 = 6.50$, 1 df, $p<0.02$). Decreased viability of group 1 pups never reached the significant level $p<0.05$. No sex effect of treatment was demonstrated.

Growth of pups was significantly delayed in treated groups with a dose-dependant effect (table 2).

Analysis of body weight data indicated a significant effect starting on the third treatment day: $F(3,36) = 7.38$, $p<0.001$. On the subsequent days, growth of treated pups was delayed in comparison with control, and the difference increased with the duration of the treatment (on the 10th treatment day: $F(3,36) = 39.28$; $p<0.001$). Scheffe's test for multiple comparisons revealed significant difference for groups 2 and 3 compared to control. Analysis revealed no sex effect of the treatments. These observations could be attributed to various causes: the treatment could decrease food comsumption of young, but the evaluation of milk ingestion by pups during the pre-weaning period is not easy. Otherwise aluminum is known to induce difficulties of absorption in young rats. Aluminum compounds inhibit gastrointestinal tract mobility (Hava and Hurwitz 1973), and delay gastric emptying in rats and man (Hurwitz et al 1976). In this way, the growth delay of treated pups could be partially attributed to a transient undernutrition.

Table 3 indicates the effects of aluminum exposure upon neuromotor development of pups.

Table 3. Neuromotor development of young rats. Median values (in parentheses: limits of interquartile ranges)

	Control	Treated Groups G1	G2	G3
G.R. (a)	72.9 (58.3-86.7)	75.0 (73.3-82.5)	65.8 (51.6-88.3)	72.1 (60 -81.6)
N.G. (b)	13.7 (10.7-28.5)	12.5 (11.0-33.7)	38.3 (21.0-51.3)	53.5 (28.3-92.8)
S.T. (c)	25.1 (16.7-28.0)	25.5 (21.3-27.0)	20.8 (15.5-23.0)	15.2 (10.5-21.3)
L.C. (d)	113.5 (60.3-119)	98.3 (64.5-121)	87.0 (78.3-141)	180.0 (161 -180)

(a): Grasping Reflex : angle (in degrees vs. horizontal line) at falling.
(b): Negative Geotaxis: time (seconds) to come back head upward.
(c): Suspension Test : time (seconds) of suspension.
(d): Locomotor Coordination : time (seconds) to put 4 feet on the platform.

Grasping Reflex data analysis revealed no difference between the groups for angular data (Kruskal-Wallis test : $H = 0.93$, 3 df, N.S.). Simultaneously proportions of rats reaching vertical position did not significantly differ (40%, 36%, 34.5%, 31.6% respectively in control and groups 1,2,3; $X2 = 0.45$, 3 df, N.S.).

At Negative Geotaxis test the ability of treated pups to turn and come back head upward was reduced in groups 2 and 3. At Kruskal-Wallis test $H = 16.36$, 3 df, $p<0.001$. At Mann-Whitney test: $U= 45.5$ (N.S.), $U = 12$ ($p<0.001$), $U = 7.5$ ($p<0.001$) respectively in comparisons control vs. group 1, group 2 and group 3.

Statistical analysis of Suspension Test data only indicated an heterogeneity between the groups at 0.10 level.(Kruskal-Wallis test : $H = 7.45$, 3 df). Mann-Whitney comparisons only indicated a significant difference between control and group 3 pups ($U = 17$, $p<0.025$).

At Locomotor Coordination test, Kruskal-Wallis test indicated a significant difference between the groups ($H = 16.42$, 3 df, $p<0.001$). Mann-Whitney test revealed no difference in the comparisons control vs. groups 1 and 2 ($U = 35$, $U = 37$, respectively), but a significant difference was detected between

control and group 3 pups ($U = 2$, $p<0.001$).

The transitory delay in neuromotor development of the pups could be partially linked to the weight delay. Otherwise, a specific attack of the Central Nervous System was not excluded: in young rats, the immaturity of the gastrointestinal tract, which enhances Aluminum absorption (Andreoli et al 1984), combined with an immature renal function (Hewitt et al 1987) might predispose treated pups to toxic levels ol Aluminum accumulation: this hypothesis will be tested in a subsequent study.

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