Bull. Environ. Contam. Toxicol. (1993) 50:85–91

Bulletin of Environmental Contamination and Toxicology

Effect of Aluminum on Lipid Peroxidation of Cerebral Hemisphere of Chick

G. B. N. Chainy, A. Sahoo, and C. Swain

Department of Zoology, Utkal University, Bhubaneswar—751 004, India

Recently much attention is being paid to aluminum due to its neurotoxicity (Boegman 1984,Macdonald and Martin 1988). Certain neurological disorders such as Amyotrophic lateral sclerosis and Parkinsonism dementia of Guam (Perl et al. 1982) and senile dementia of Alzheimer type (Crapper et al. 1973) were attributed to Al toxicity. There are evidences that Al ingestion induces behavioral toxicity in mice (Golub et al. 1989), rat (Thorne et al. 1986) and rabbit (Yokel 1989). Lipid peroxidation has been proposed as a mechanism of cellular membrane damage during heavy metal induced toxicity (Stacey and Kappus 1982). Brain being rich in polyunsaturated fatty acids is highly susceptible to lipid peroxidation (Sun and Sun 1974). The present study was designed to investigate the effect of Al ingestion on lipid peroxidation as well as on free sulfhydryl levels in cerebral hemisphere (CH) of chick. In addition we have also studied the effect of Al on lipid peroxidation in CH of 1 day and 56 day old chicks in vitro in order to know whether Al is capable of inducing lipid peroxidation and if so, whether such induction is age dependent or not.

MATERIALS AND METHODS

1 day and 56 day old male white leg horn chicks, obtained from Central Government Poultry Farm situated locally, were used for in vitro study. For Al ingestion study, 1 day chicks were maintained in the laboratory aviary with 24 h light period. They were fed commercial chick food and water was supplied ad libitum. Twenty two chicks of age 1 day were divided into three groups. Group II and III were fed orally 200 and 400 mg $Al_2(SO_4)_3.18H_2O$/Kg body wt. in 0.1 ml volume of distilled water for 15 days respectively. Birds of Group I received vehicle solution and served as control for Group II and III. All birds were killed 24 h after the last treatment. Cerebral hemisphere was dissected out, cleaned in normal saline solution and homogenized in ice-cold 1.15% KCl solution (10%, w/v) with the help of a motor-driven glass-teflon homogenizer and used for

Send reprint requests to G.B.N.Chainy.

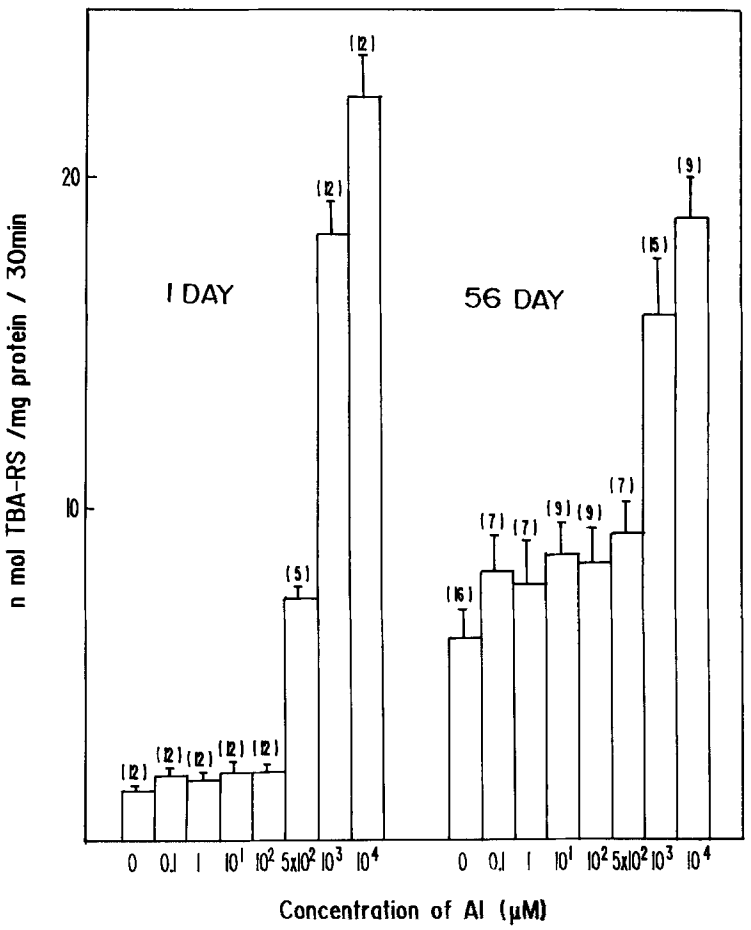

Figure 1. Concentration dependent effects of $Al_2(SO_4)_3$ on lipid peroxidation in CH homogenates of 1 day and 56 day old chicks. Data are mean ± SEM of number of observations given in the parentheses.

lipid peroxidation. Homogenates of the CH obtained from 1 day and 56 day were used to study the effect of Al on lipid peroxidation in vitro. To test the effect of Al, tissue homogenates (0.2 ml) containing approximately 1 mg protein were incubated with different concentrations of aluminum sulphate for various time intervals in a shaking water bath at 37°C. Appropriate controls were used for each set. After the end of the incubation time lipid peroxidation was measured.

Lipid peroxidation was estimated by thiobarbituric acid (TBA) assay (Ohkawa et al. 1979). Sensitivity of tissue homogenates of Al ingested birds to in vitro oxidation was estimated by

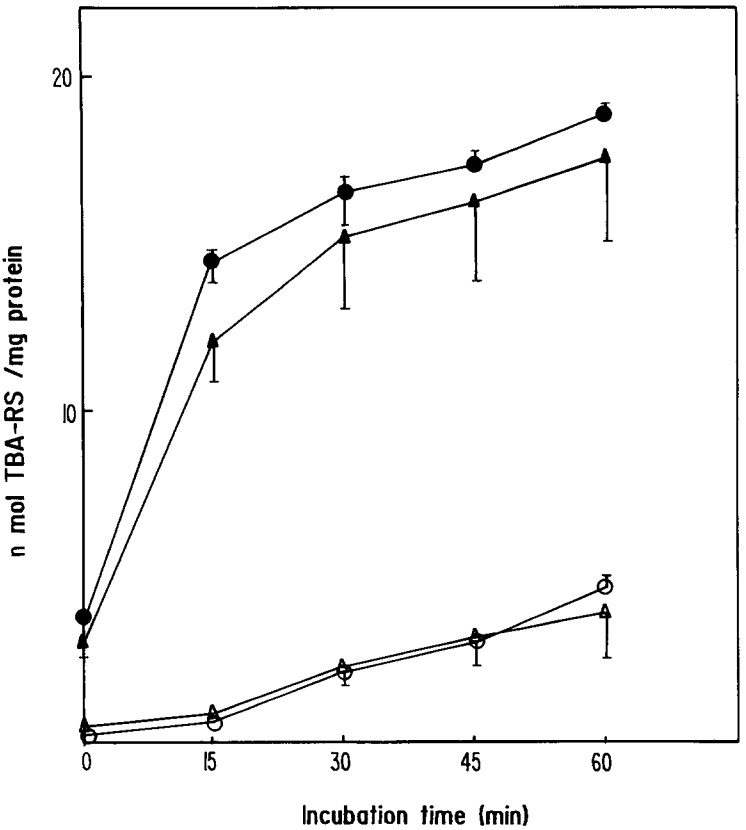

Figure 2. Effects of incubation time with $Al_2(SO_4)_3$ (final conc. ImM) on lipid peroxidation in CH homogenates of 1 day (●) and 56 day (▲) old chicks. Data are mean ± SEM (n = 4). ○ and △ are respective age group controls.

incubating homogenates with 0.05 mM ascorbic acid and 0.1 mM $FeSO_4$ (final conc.) in a shaking water bath at 37°C prior to performing of TBA assay. The concentration of TBA-reactive substances (TBA-RS) was calculated from extinction coefficient of 156 M^{-1} cm^{-1} (Wills 1969). Results were expressed as nmole malonaldehyde formed per mg protein. Free sulfhydryl level (-SH) in the tissues were estimated (Ellman 1959). The protein concentration was determined by Lowry et al. (1951). $Al_2(SO_4)_3.18H_2O$ was procured from E. Merck and solution was prepared in distilled water. This batch of $Al_2(SO_4)_3.18H_2O$ has 0.005% iron contamination. Data were analysed for the

significance of difference using Student's t test and were considered statistically significant when P value was < 0.05.

RESULTS AND DISCUSSION

Formation of TBA-RS is widely used as an index of extent of lipid peroxidation. Fig. 1 shows that CH homogenate from 56 day old chick produced more TBA-RS than 1 day old chick when incubated aerobically. Al concentration dependent lipid peroxidation was evident only above 100 µM and 500 µM in 1 day and 56 day old chicks respectively. At higher concentration Al-induced lipid peroxidation was more or less of same magnitude for both the age groups. Measurement of change in TBA-RS production with time during incubation at 37°C (Fig. 2) showed that there were no significant difference in TBA-RS production by homogenates of 1 day and 56 day old chicks till 60 min of incubation with Al (final conc. 1 mM). Time course study revealed that formation of TBA-RS was evident within 15 min of incubation with Al and attained peak level at 30 min and remained constant thereafter upto 60 min. Pilot experiments showed that under 30 min of incubation, formation of TBA-RS in response to 1 mM Al was linearly positive in relation to protein concentration (a=1.94;b=6.82; r=+0.77; n=10).

The exact mechanism by which Al induced formation of TBA-RS in crude homogenates of CH in vitro is not clear from this study. At the present stage, however, we assume that Al has some influence on membrane lipids or other organic molecules of the cell and thus facilitates lipid peroxidation. Polyunsaturated fatty acids are abundantly found in brain and are highly susceptible to peroxidation (Sun and Sun 1974). Lipid peroxidation is believed to be an indicator of membrane damage resulting from the degradation of polyunsaturated fatty acids (Tam and McCay 1970). Al is also known to alter physical properties of the membrane (Vierstra and Haug 1978). Histochemical investigations have revealed the association of Al with nuclear chromatin of the brain (DeBoni et al. 1974) and in vitro studies have confirmed the formation of complexes of Al with DNA (Karlik et al. 1980; Karlik and Eichhorn 1989). A selective binding of Al to phosphotidylserine, a major phospholipid of the brain has been also reported (Blaustein 1967). It has been shown that free radical damage to polyunsaturated fatty acids, carbohydrates, amino acids and nucleic acids have resulted in elevation of TBA-RS mainly because of generation of malonaldehyde (Gutteridge 1982).

Ingestion of Al for 15 days to 1 day old chicks had no significant effect on body weight, brain weight, and height and length of the comb (Table 1). Endogenous level of TBA-RS were also similar in both control and Al treated chicks. Incubation of tissue samples with ascorbic acid and $FeSO_4$ for 60 min had resulted in significant elevation in TBA-RS values than the respective in vivo data. However, the magnitude of increase was more or less of same value in control and Al treated groups.

Table 1. Effect of Aluminum sulphate ingestion on various parameters of the chick.

Parameters	Control (12)	Aluminum salt treated 200 mg (5)	Aluminum salt treated 400 mg (5)
Body wt. (g)	122.67 ± 18.88	115.20 ± 37.45	112.00 ± 10.37
Cerebral hemisphere wt. (g)	0.76 ± 0.03	0.76 ± 0.07	0.75 ± 0.03
Comb length (cm)	1.72 ± 0.20	1.75 ± 0.19	1.50 ± 0.15
Comb height (cm)	0.58 ± 0.15	0.63 ± 0.10	0.52 ± 0.10
Cerebral hemisphere			
Protein content (mg/g wet wt.)	118.44 ± 13.20	119.41 ± 17.59	118.04 ± 14.73
-SH content (nmol/mg protein)	14.16 ± 2.92	11.86 ± 3.20	14.13 ± 3.80
Lipid peroxidation (nmol MDA/mg protein)			
Without $FeSO_4$ and ascorbic acid	0.55 ± 0.12	0.49 ± 0.23	0.63 ± 0.26
With $FeSO_4$ and ascorbic acid	15.48 ± 1.62	16.10 ± 2.12	15.17 ± 0.71

Data are expressed as mean ± SD of number of observations in the parentheses.

Also Al ingestion had no effect on free -SH group and protein contents of CH from that of control ones (Table 1).

It is possible that dose and duration of treatment of Al in the present study may not be sufficient to evoke lipid peroxidation under in vivo condition. It is reported that there is differential tissue uptake of Al when ingested as a variety of organic and inorganic salts (Greger et al. 1985; Slanina et al. 1985). Alternatively, it is also possible that chicks particularly young ones may be more resistant to Al toxicity since Yokel (1989) has reported that Al induced behavioral toxicity was more in adult and aged rabbits than in young ones.

Acknowledgments. We are thankful to Head, Department of Zoology, Utkal University, Bhubaneswar for providing necessary laboratory facilities.

REFERENCES

Blaustein MP (1967) Phospholipids as ion exchangers: Implications for a possible role in membrane excitability and anaesthesia Biochim Biophys Acta 135:653-668.

Boegman RJ, Bates LA (1984) Neurotoxicity of aluminum. Can J Physiol Pharmacol 62:1010-1014.

Crapper DR, Krishnan SS, Dalton AJ (1973) Brain aluminum distribution in Alzheimer's disease and experimental neurofibrillary degeneration. Science 180:511-513.

DeBoni U, Scott JW, Crapper DR (1974) Intracellular aluminum binding, a histochemical study. Histochemistry 40:31-37.

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77.

Golub MS, Donald JM, Gershwin ME, Keen CL (1989) Effect of aluminum ingestion on spontaneous motor activity of mice. Neurotox and Teratol 11:231-235.

Gutteridge JMC (1982) Free-radical damage to lipids, amino acids, carbohydrates and nucleic acids determined by thiobarbituric acid reactivity. Int J Biochem 14:649-653.

Greger JL, Bula EN, Gum ET (1985) Mineral metabolism of rats fed moderate levels of various aluminum compounds for short periods of time. J Nutrition 115:1708-1716.

Karlik SJ, Eichhorn GL, Lewis PN, Crapper DR (1980) Interaction of aluminum species with deoxyribonucleic acid. Biochemistry 19:5591-5598.

Karlik SJ, Eichhorn GL (1989) Polynucleotide cross-linking by aluminum. J Inorg Biochem 37:259-269.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-275.

Macdonald TL, Martin RB (1988) Aluminum ion in biological systems. Trends in Biochem Sci 13:15-19.

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351-358.

Perl DP, Gajdusek DC, Garruto RM, Yanagihara RT, Gibbs CJ (1982) Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. Science 217: 1053-1055.

Slanina P, Frech W, Berhardson A, Cedergren A, Mattson P (1985) Influence of dietary factors on aluminum absorption and retention in the brain and bones of rats. Acta Pharmacol Toxicol 56:331-336.

Stacey NH, Kappus H (1982) Cellular toxicity and lipid peroxidation in response to mercury. Toxicol Appl Pharmacol 63:29-35.

Sun GY, Sun AY (1974) Synaptosomal plasma membranes: acyl group composition of phosphoglycerides and Na^+, K^+-ATPase activity during fatty acid deficiency. J Neurochem 22:15-18.

Tam BK, McCay PB (1970) Reduced triphosphopyridine nucleotide oxidase-catalyzed alterations phospholipid peroxides. J Biol Chem 245:2295-2300.

Thorne BM, Donohoe T, Lin K-N, Lyon S, Mediros DM, Weaver ML (1986) Aluminum ingestion and behavior in Long-Evans rat. Physiol Behav 36:63-67.

Wills ED (1969) Lipid peroxide formation in microsomes. General considerations. Biochem J 113:315-324.

Vierstra R, Haug A (1978) The effect of Al^{+3} on the physical properties of membrane lipids in Thermoplasma acidophilium Biochem Biophys Res Comm 84:138-143.

Yokel RA (1989) Aluminum produces age related behavioral toxicity in the rabbit. Neurotox Teratol 11:237-242.

Received February 26, 1992; accepted July 20, 1992.