Journal of Animal and Veterinary Advances 11 (19): 3588-3592, 2012
ISSN: 1680-5593

Does Selenium Ameliorate Toxic Effects of Prenatal Aluminium on Brain of Full Term Rat Fetuses?

[1]Samar M. Al Saggaf, [1,3]Ghada A. Abdel-Hamid, [2]Magda Hagras and [1]Hamid A. Saleh
[1]Department of Anatomy, [2]Department of Pharmacology,
Faculty of Medicine, King Abdul Aziz University, Jeddah, KSA
[3]Department of Anatomy, Faculty of Medicine, Suez Canal University, Ismaillia, Egypt

Abstract: Aluminum (Al) has been implicated in the pathogenesis of dialysis dementia and Alzheimer's diseases. The present study aimed to investigate the protective effect of selenium on fetal parietal cortex treated with aluminum chloride. Eighty pregnant rats were divided into two groups (Control and Aluminium). Aluminium treated group was given oral $AlCl_3$ (150 mg kg^{-1} body weight/day) for 3 months before mating. Pregnant rats were randomly divided in to four groups (20 dams in each), control, Al-treated, control + Selenium (200 μg/kg/day) and Al + Selenium. Al and Selenium was administered through intragastric tube from GD1 to GD20. All growth parameters significantly reduced in all Al-treated groups. $AlCl_3$ induced increase thickness of pia matter and increase glia fibers in the molecular layer, disruption, shrinkage and degeneration of neurons in all cortical layers. Parietal cortex in fetuses treated with selenium and $AlCl_3$ had nearly control appearance. Also, $AlCl_3$ induced significant reduction of layer I and the total thickness of parietal cortex. Selenium when added to $AlCl_3$ significantly reduced all harmful effect of $AlCl_3$ on fetal growth parameters, histopathological changes and increased thickness of layer I of parietal cortex in rat fetuses when compared with control. It is concluded that oral $AlCl_3$ had deleterious effects on cerebral cortex of rat fetuses whereas selenium alleviated these negative effects.

Key words: Aluminium, fetus, selenium, brain, neurons

INTRODUCTION

Aluminum (Al) is a widely distributed metal in the environment and is extensively used in modern daily life. Al enters into the body from the environment and from diet. Al containing diet is mainly corn, yellow cheese, salt, herbs, spices, tea and cosmetics (Yousef, 2004). Moreover, Al is incorporated in some medications such as antacids, buffered aspirins and anti-diarrheal products. Al sulfate is extensively added as a coagulant agent during the purification process of drinking water in order to folliculate the organic matter to clarify the water (Ochmanski and Barabasz, 2000).

Nervous system is a vulnerable target for toxicants due to critical voltages which must be maintained in the cells and the all responses when voltages reach threshold levels. Aluminum has the potential to be neurotoxic in human and animals. Oral exposure to Al may result in accumulation of Al in hippocampus of brain and thus affect some essential elements (Zn, Fe, Cu and Ca) contents in the hippocampus at different degrees (Yang *et al*., 2002; Jia *et al*., 2001). Selenium is generally recognized to be a trace mineral of great importance for human health which protects the cells from the harmful effects of rancidity. Selenium counteracts cancer and chromosome damage as well as increases the resistance to viral and bacterial infections (Hoffman and Heinz, 1998; Yuan and Tang, 1999).

Although, some studies examined the toxic effects of Al containing substances on matured animal models (Abbasali *et al*., 2005; Bihaqi *et al*., 2009; El-Demerdash, 2001, 2004; Hussein *et al*., 2010; Deloncle *et al*., 1999) little attention was paid to prenatal toxic effect of Al on fetuses. It should be kept in mind that many toxic compounds that may be tolerable in certain concentrations in adults are harmful for fetuses. Therefore, the present study was carried out to investigate the efficiency of selenium in altering the oxidative damage and neurotoxic effects of aluminum of full term fetuses rats.

MATERIALS AND METHODS

Animals and experimental design: This study was performed on eighty Virgin Albino rats Sprague Dawley rats weighing between (150-180 g) were purchased from

Corresponding Author: Ghada A. Abdel-Hamid, Department of Anatomy, Faculty of Medicine, King Abdul Aziz University, P.O. Box 42806, 21551 Jeddah, KSA

animal house in King Fahd Medical Research Center. The experimental design was approved by the local committee and was conformed to the guidelines of National Institute of Health (NIH). The animals were housed in stainless steel cages and maintained on a 12 h light-dark cycle and 27±1°C room temperature and hygienic conditions. Water was offered *ad libitum*. Rats were randomly divided into two main groups: Control (C) and Aluminum (Al). Al group was given oral $AlCl_3$ (150 mg kg^{-1} body weight/day) for 3 months before mating.

Breeding: One fertile male Albino rat was introduced into a cage with two females and remained there overnight. Pregnancy was determined by the presence of spermatozoa in the vaginal smear next morning and this was considered the Ist day of Gestation (GD1). Once pregnancy occurs each main group was be divided into two subgroups, 20 female rat in each:

Control:
- (C) untreated (distilled water)
- (C) + Se (200 μg/kg/day)

Aluminum:
- $AlCl_3$ (150 mg/kg/day)
- $AlCl_3$ (150 mg/kg/day) + Se (200 μg/kg/day)

$AlCl_3$ and selenium was administered through intragastric tube from GD1 to GD20. At the same time control groups were adminstried distilled water through intragatric tube.

Chemicals: Aluminium chloride ($AlCl_3$) and sodium selenite (Na_2SeO_3) used in this study were purchased from Aldrich Chemical Company (Milwaukee, USA). Selenium and all other used chemicals were purchased from Sigma Chemical Co. (St. Louis, USA).

Samples and parameters measured: At the time of sacrifice (GD20) dams were killed by overdose of ether. The abdomen of the dams was be opened to extract the fetuses from the uterine horns. Implantation sites was be counted and compared with the numbers of corpcra lutea to detect preimplantation losses:

$$\text{Preimplantation loss} = \text{Number of corpora lutea} - \text{Number of implantation sites}$$

$$\text{Resorpition} = \text{Number of implantation sites} - \text{Number of fetuses}$$

The following growth parameters for each fetus was taken: crown rump length, body weight, biparietal diameter and head length. The vault of the cranium was removed and the brain was be exposed and dissected out carefully, fetal brains were removed then were fixed in formaline 10% and proceed for paraffin sections. Sections (median sagittal) of 4 μm thickness in parietal cortex were prepared and stained with Haematoxylin and eosin stain (Banchroft *et al*., 1996; Kiernan, 2000). Thickness of cerebral cortex and their molecular layers were measured using Image pro-plus program.

Statistical analysis: The values of the various parameters (fetal weight, brain weight, head length, biparietal diameter and cerebral cortex thickness) were calculated for each rat and then pooled to obtain the mean and standard deviation for all rats within a given group. Differences between groups were tested by a paired Student t-test. All differences were considered as statistically different at $p<0.05$.

RESULTS AND DISCUSSION

Effect of aluminium and selenium on female fertility: Oral administration of $AlCl_3$ reduced pregnancy rate of female rats (70%) in comparison with control group (100%). Oral administration of selenium with $AlCl_3$ increase pregnancy rate in female rats (80%). In $AlCl_3$ and $AlCl_3$+Se groups, significant decrease ($p<0.05$) in number of corpora lutea, implantation sites, viable fetuses when compared with control and C+Se groups respectively (Table 1). On other hand, $AlCl_3$ significantly increased ($p<0.05$) pre-implantation loss and number of resorption in comparison with control group (Table 1).

Morphological and growth parameters: No growth morphological congenital anomalies in fetuses in all groups. All growth parameters included body weight, brain weight, Crown Rump Length (CRL), Biparietal Diameters (BPD) and Head Length (HL) were significantly

Table 1: Effect of aluminum chloride on fertility of adult female rats

Groups	No. of males	No. of pregnant females	No. of corpora lutea	No. of implantation sites	No. of viable fetuses	Pre-implantation loss	Resorption
C	5	10 (100%)	9.6±1.6	8.7±0.96	8.2±0.6	0.20±0.40	0.05±0.2
Al	5	7 (70%)	5.2±2.1*	4.9±1.70*	4.4±1.3*	3.30±1.10*	4.10±0.6*
C+Se	5	10 (100%)	8.5±1.1	8.1±0.79	7.8±0.8	0.25±0.40	0.15±0.4
Al+Se	5	8 (80%)	6.5±1.3*	6.0±0.86*	5.7±0.6*	1.00±0.60*	0.70±0.6*

*Student t-test; $p<0.05$ compared to corresponding control group; Data are means±standard deviation; The ±values represent the mean±SD

($p<0.05$) less in the $AlCl_3$ group compared with control. No significant difference in all growth parameters between C+Se and $AlCl_3$+Se groups (Table 2).

Histopathological findings: In full term fetuses, neurons in parietal cortex appeared small closely packed, rounded to oval cells with active vesicular nuclei. Layer I (molecular layer) was the only layer which could be distinguished from other layers (II-VI) (Fig. 1 and 2). In $AlCl_3$-treated group pia matter was thickened, increase glia fibers in molecular layer. Neurons in all cortical layers were disrupted, shrinkaged and degenerated with increase spaces between them. Selenium when was administrated to rats with $AlCl_3$, ameliorated all histopathological changes in fetal parietal cortex induced by $AlCl_3$. Therefore, histological appearance of parietal cortex in fetuses treated with $AlCl_3$+Se was very close to control+Se (Fig. 3).

Morphometric measurements: The total thickness of parietal cortex and thickness of layer I decreased significantly ($p<0.05$) in $AlCl_3$ group in comparison with control (Table 3). Layer I in $AlCl_3$+Se (34.8±4.8) was increased significantly ($p<0.05$) when compared with control+Se group (28.1±1.6) (Table 3).

Table 2: Prenatal effects of aluminum chloride and selenium on growth parameters of full term rat fetuses

Groups	Fetal wt (g)	Brain wt (g)	CRL (cm)	HL (cm)	BPD (cm)
C	2.60±0.28	0.080±0.06	3.0±0.40	1.35±1.16	0.69±0.11
Al	2.50±0.22*	0.060±0.01*	2.5±0.10*	1.16±0.08*	0.62±0.04*
C+Se	2.90±0.12	0.082±0.01	2.7±0.26	1.26±0.08	0.65±0.06
Al+Se	2.92±0.10	0.084±0.01	2.2±0.35	1.10±0.10	0.64±0.07

*Student t-test; $p<0.05$ compared to corresponding control group; Data are means±standard deviation

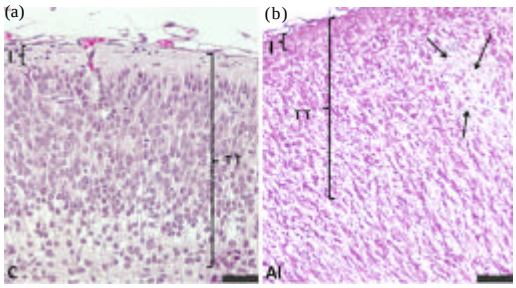

Fig. 1: Section in parietal cortex of (a) Control (C) and (b) Aluminium (Al) groups showing normal architecture of parietal cortex in control group while loss of these in Al group. Notice area of degeneration (arrows). Thickness of layer I and total thickness of parietal cortex are less in Al group than control. I: molecular layer, TT: Total Thickness. Original magnification is x400, scale bars = 50 μm (H&E)

Table 3: Prenatal effects of Aluminum chloride and selenium on cortical parameters in full term rat fetuses

Thickness (μm)	C	Al	C+Se	Al+Se
Total cerebral cortex thickness	183.4±4.4	167.4±4.30*	268.7±7.7	242.1±5.0*
Thickness of molecular layer	21.6±4.3	16.7±6.00*	28.1±1.6	34.8±4.8*

*Student t-test; $p<0.05$ compared to corresponding control group; Data are means±standard deviation; The ±values represent the Mean±SD

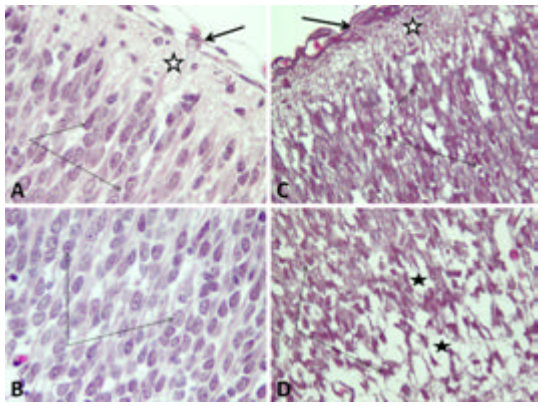

Fig. 2: Sections in parietal cortex of rat: A and B (control) showing normal pia covering (thick arrow); molecular layer glia cells and fibers (white star). Neuronal cell layers showed rounded to oval cells with active vesicular nuclei (thin arrows). C and D (Aluminum group) showing thick pia matter (thick arrow). Increase glia fibers in molecular layer (white star). Neuronal cell layer showed degenerated ill-defined neurons with dark stained degenerated nuclei (thin black arrows). Spaces (black stars) in between were created due to marked cell shrinkage and degenerations (H&E x1000)

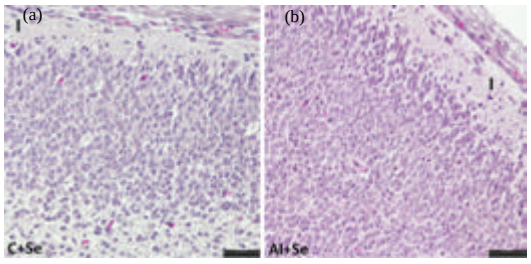

Fig. 3: Section in parietal cortex of (a) C+Se and (b) Al+Se groups showing normal architecture of parietal cortex in both groups. Thickness of layer I of parietal cortex are more in (AL+Se) group than (C+Se). I: molecular layer. Original magnification is x400, scale bars = 50 μm (H&E)

The role of heavy metals in the pathogenesis of neurodegenerative disease is currently receiving considerable attention (Singh, 2004). Aluminum is present in many manufactured foods and medicines and is added to drinking water for purification purposes. It has been proposed that aluminum is a contributing factor to several neurodegenerative disorders such as Alzheimer's disease (Ribes *et al*., 2008). However, this remains controversial primarily due to the unusual properties of aluminum and a lack of information on its cellular sites of action. In this study, protective effect of oral selenium on neurotoxicity of $AlCl_3$ on full term rat fetuses was investigated. In the present research, oral administration of $AlCl_3$ to adult female rats was reduced pregnancy rate. In $AlCl_3$ and $AlCl_3$+Se groups, significant decrease in pregnancy rate, number of corpora lutea, implantation sites, viable fetuses while increased significantly pre-implantation loss and number of resorption when compared with control and C+Se groups, respectively. Resorption may indicate transplacental passage of $AlCl_3$ to embryo beside to modification of the uterine lining which restricted the development of the implanted embryos. These findings were in accordance with Elbetieha *et al*. (2008) who reported that $AlCl_3$ induced a significant decrease in pregnancy rate and a significant increase in number of female rats with resorptions.

The results presented in this study, regarding the growth of the rat fetuses, revealed that all growth parameters included body weight, brain weight, crown rump length biparietal diameters and head length were significantly less in the $AlCl_3$ group compared with control. These findings were in agreement with Abbasali *et al*. (2005) who reported that intraperitonal injection of $AlCl_3$ (150 mg kg^{-1} to pregnant on the 10th, 11th and 12th days of gestation significantly reduced the fetal body weight and crown rump. Aluminum ion (Al^{+3}) is a trivalent cation and has a high affinity for negatively charged groups. It has been proposed that Al preferentially interacts with phosphate groups such as nucleic acids and phosphorylated proteins. In this way, Al remarkably decreases DNA and RNA synthesis and inhibits embryonic cell proliferation and protein synthesis (Nicholls *et al*., 1995; Yumoto *et al*., 2001). This mechanism can explain the toxic effects of Al on growth of the embryo and fetus.

In the present research, oral administration of $AlCl_3$ to pregnant rat induced, increase thickness of pia matter and increase glia fibers in the molecular layer, disruption, shrinkage and degenration of neurons in all cortical layers. Parietal cortex in fetuses treated with selenium and $AlCl_3$ had nearly control appearance. So, selenium reduced the deleterious effects of $AlCl_3$ on neurons of parietal cortex in rat fetuses. Parallel to the histopathological findings, those mentioned by Hussein *et al*. (2010) and Bihaqi *et al*. (2009) who stated that oral $AlCl_3$ causes histopathological lesions in cerebral cortex of adult male rats including neuronal degeneration as cytoplasmic vacuolization hemorrhage, ghost cell and gliosis. Agrawal *et al*. (1995) reported that $AlCl_3$ exert its toxicity including increasing the blood-brain barrier permeability, interference with phosphorylation-dephosphorylation reactions, altered iron metabolism with subsequent free-radical production and disruption of second messenger systems.

Regarding the total thickness of parietal cortex and thickness of layer I $AlCl_3$ resulted in significant reduction their thicknesses. On the other hand, selenium when added to $AlCl_3$ significantly increased thickness of layer I of parietal cortex in rat fetuses when compared with control. The present data showed that the presence of selenium with $AlCl_3$ alleviated its harmful effect on all above measured parameters and their levels become near to the normal values of control. All the above findings in this study, regarding the protective effects of selenium against neurotoxic effects of $AlCl_3$ can be explained by the results of Yuan and Tang (1999) who reported that selenium is has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against the injury of oxidation.

El-Demerdash (2004) stated that selenium in combination with Al significantly decreased level of free radicals and increased the activity of glutathione S-transferase in brain. The protective effect of selenium against the neurotoxic effect of $AlCl_3$ may be attributed to the ability of selenium to appear as the first line of defense against peroxidation of polyunsaturated fatty acid contained in cellular and subcellular membrane phospholipids.

CONCLUSION

The present study demonstrated that selenium in combination with $AlCl_3$ minimized its harmful effect. Selenium counteracts $AlCl_3$ harmful effects by preventing free radical formation. Consequently, efforts must be done to reduce the exposure to aluminum as well as using diet rich in selenium may be beneficial in alleviating aluminum toxicity.

ACKNOWLEDGEMENTS

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Under Grant No. (24/428). The researchers therefore acknowledge with thanks DSR technical and financial support.

REFERENCES

Abbasali, K.M., T. Zhila and N. Farshad, 2005. Developmental toxicity of aluminum from high doses of AlCl3 in mice. J. Applied Res., 5: 575-579.

Agrawal, S.K., L. Ayyash, C.S. Gourley, J. Levy, K. Faber and C.L. Hughes, 1995. Evaluation of developmental neuroendocrine and reproductive toxicology of aluminum. Food Chem. Toxicol., 34: 49-53.

Banchroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and Practice of Hisological Techniques. 4th Edn., Churchil Livingstone, New York, London, San Francisco, Tokyo.

Bihaqi, S.W., M. Sharma, A.P. Singh and M. Tiwari, 2009. Neuroprotective role of Convolvulus pluricaulis on aluminium induced neurotoxicity in rat brain. J. Ethnopharmacol., 124: 409-415.

Deloncle, R., F. Huguet, P. Babin, B. Fernandez, N. Quellard and O. Guillard, 1999. Chronic administration of aluminium L-glutamate in young mature rats: Effects on iron levels and lipid peroxidation in selected brain areas. Toxicol. Lett., 104: 65-73.

El-Demerdash, F.M., 2001. Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver and blood of rats. J. Environ. Sci. Health B., 36: 489-499.

El-Demerdash, F.M., 2004. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminum. J. Trace Elem. Med. Biol., 18: 113-121.

Elbetieha, A., A. Shoter, A. Mohammed, I. Mayyas, W. Khamas and Z. Elnasser, 2008. Toxicity evaluation of aluminium chloride on adult female mice. J. Anim. Vet. Adv., 7: 552-556.

Hoffman, D.J. and G.H. Heinz, 1998. Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. Environ. Toxicol. Chem., 17: 161-166.

Hussein, M., A. Abd-El-Rahman and E.T. Mohamed, 2010. The protective effect of vitamin e against the neurotoxic effect of aluminum cholorid in male albino rat. J. Am. Sci., 6: 978-991.

Jia, Y., C. Zhong and Y. Wang, 2001. Effects of aluminum on amino acid neurotransmitters in hippocampus of rats. Zhonghua Yu Fang Yi Xue Za Zhi, 35: 397-400.

Kiernan, J.A., 2000. Histological and Histochemical Methods: Theory and Practice. 3rd Edn., Butterworth-Heinemann, Oxford, Boston, Johannesburg, New Delhi, Pages: 502.

Nicholls, D.M., G.M. Speares, S. Asina and A.C.M. Miller, 1995. Brain mRNA from infants of aluminium-exposed lactating rabbits. Int. J. Biochem. Cell Biol., 27: 365-370.

Ochmanski, W. and W. Barabasz, 2000. Aluminium-occurrence and toxicity for organisms. Przegl. Lek., 57: 665-668.

Ribes, D., M.T. Colomina, P. Vicens and J.L. Domingo, 2008. Effects of oral aluminum exposure on behavior and neurogenesis in a transgenic mouse model of Alzheimer's disease. Exp. Neurol., 214: 293-300.

Singh, M., 2004. Role of micronutrients for physical growth and mental development. J. Pediatr., 71: 59-62.

Yang, J., Y. Jia, R. Zhao, N. Jin and J. Chen, 2002. Effects of exposure to aluminum on some metal elements contents in hippocampus of rat. Zhonghua Yu Fang Yi Xue Za Zhi, 36: 247-249.

Yousef, M.I., 2004. Aluminum-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: Protective role of ascorbic acid. Toxicology, 199: 47-57.

Yuan, X. and C. Tang, 1999. Lead effect on DNA and albumin in chicken of selenium nutrition. J. Environ. Sci. Health A., 34: 1875-1887.

Yumoto, S., H. Nagai, H. Matsuzaki, H. Matsumura, W. Tada, E. Nagatsuma and K. Kobayashi, 2001. Aluminium incorporation into the brain of rat fetuses and sucklings. Brain Res. Bull., 55: 229-234.