Proceedings S.Z.P.G.M.I vol: 14(2) 2000, pp. 85-90.

Effect of Aluminium on the Crown-Rump Length of Embryo in Mice

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SUMMARY

In order to determine the embryotoxicity and teratogenicity of aluminium containing compounds present study was carried out. 72 pregnant mice were given a daily intraperitoneal dose of 0.7 mg/100 mg of aluminium sulphate for different periods. This dose was equivalent to maximum therapeutic dose of aluminium salt for a 70 kg man i.e. 5000 mg aluminium/day. Fetal examination was performed on day 20 of gestation. The number of live and dead fetuses in the treated animals were not significantly different from the control groups. Therefore embryolethality of aluminium cannot be deduced. However there was a decrease in fetal body weight and crown-rump length that was directly related to the duration of exposure to aluminium sulphate solution.

INTRODUCTION

There is no dispute that maternal intoxication with certain metals in both man and laboratory animals may adversely affect pregnancy and the development of conception. Until first half of the 20th century, it was assumed that the development of embryo was dependent entirely on hereditary factors, but the observation made by Gregg in 1941 regarding association of congenital cataract with rubella infection in pregnancy opened up a new field of research in human development defects as a result of exposure to environmental factors[1].

The earth surface is abundant in aluminium compounds and there is growing realization that excessive exposure to aluminium can produce aluminium accumulation and toxic syndromes under certain conditions[2].

Aluminium is widely distributed in nature. It is found in both organic and inorganic forms in vegetation and in all vertebrate species[3]. Its level depends upon the geochemistry of local environment. In municipal water supplies slightly higher levels are found because sulphate is used during water purification procedures[4]. Its wide distribution in nature and increasing use of this metal for cooking utensils from which small amounts may be dissolved by the food, especially in the presence of alkalis and sodium chloride has stimulated various workers to study its influence on animals, plants and human beings. Daily dietary intake of aluminium varies from country to country because of wide variation in the dietary habits of individuals. An average European or American adult consumes 20-30 mg aluminium daily in foods and beverages. In these countries high dietary aluminium content is due to common use of aluminium containing food additives, food dyes and colours, in preparation of baked, processed and canned foods[5]. In African and Asian countries e.g. Pakistan, the major source of dietary aluminium is cooking utensils. It has been found that most foods stored or cooked n aluminium pans, trays or foils accumulate some aluminium. Aluminium content of foods cooked or baked in aluminium utensils depends upon various factors such as pH length of cooking period, usage of new pans or pressure cooker etc. 2-3 mg of aluminium may be ingested daily from a normal routine diet. As a rule 6 mg/day is considered as an average daily intake of aluminium in diet[6].

As the aluminium is abundantly present in earth's crust, humans are continuously exposed to this element. The lungs, skin and gastrointestinal tract are major barriers to aluminium absorption, which is limited to only few micrograms per day.

The small amount of aluminium that is absorbed from the body is readily eliminated from the body by the kidneys. Thus is healthy individuals, under normal circumstances, with normal exposure, there is little evidence that aluminium offers any risk to the individual. However absorption barriers can be over come when large doses of aluminium are given orally in the form of antacids[7] or when aluminium is given parenteraly[8].

In the body it has a number of biological effects. It promotes the reaction between cytochrome C and succinic delydrogaose and is a necessary co-factor for the acceleration of guanine nucleotide binding protein by fluoride for the stimulation of adenylate cyclase activity[9]. It has an inhibitory effect on bone-phosphates, hexokinases[10,11] and enhances the activity of cholinesterase[10]. Aluminium has also been shown to displace magnesium from ATP[11]. The resulting stabilization of ATP prevents phosphate transfer by Na^+ - K^+ - ATPase[10]. It also binds calmodulin[3] and inhibits ferroxidase (ceruloplasmin) activity[7]. Apart from its important biological actions, a number of syndromes are associated with aluminium toxicity e.g. Alzheimer disease[12]. Amytrophic lateral sclerosis and Parkinsonian dementia of Guamn[13]. In experimental animals it has also been found to be nephrotoxic[14]. Maternal toxicity during pregnancy was evaluated by Cranmer[16]. Bennet[15] found aluminium highly teratogenic in rats with obvious skeletal defects and growth retardation. McCormak[17] reported no significant effect on resorption rate or incidence of soft tissues skeletal deformities. Suggesting that aluminium might not be teratogenic in rats.

The present study was carried out to establish the role of aluminium containing compounds in producing embryotoxic and teratogenic effects.

MATERIAL AND METHODS

Seventy two female and 36 male albino mice were used for the present study. Animals were kept (in the animal house of Postgraduate Medical Institute, Lahore) in separate cages and fed with commercially prepared chick-feed No.3 and water *ad libitum*. Optimum light and temperature was maintained in the animal room. Prior to the start of the experiment, animals were kept for 14 days without treatment to exclude any already pregnant females.

Mating was allowed in dark during the estrus period. Vaginal smear study was not done to avoid pseadopregnancy. Presence of vaginal plug was considered as a sign of conception and the day was taken as day 1 of pregnancy.

The salt of aluminium used for the present study was $Al_2(SO_4).16H_2O$. Aluminium sulphate is soluble in water, so the aqueous solution was used for the project. The dose was calculated in such a way that the required amount of aluminium was present in 0.25 ml of aqueous solution of aluminium sulphate. 0.7 mg/100 gm body weight was the required amount which was calculated as follows. The maximum therapeutic dose of aluminium salt for a 70 kg man is 5000 mg/day, 10% of this is absorbed from gastrointestinal tract[7] which is 0.713 mg/100 gm/day. The animals were weighed and average weight was found to be 50 mg.

Route of administration

Drug was administered intraperitoneally starting from the day 1 of the pregnancy for various periods according to the grouping.

Experimental design

Pregnant female mice were divided at random into various control and experimental groups, labeled and given intraperitoneal injections of drug accordingly.

Control group

The animals of control group were further divided into subgroups A, B, C, D, E and F each comprising 6 pregnant animals. Each mouse was given daily intraperitoneal injection of distilled water for the periods given in Table 1 and sacrificed on day 20 of gestation.

Experimental group

The animals of experimental group were also divided into sub-groups A1, B1, C1, D1, E1 and F1, each comprising 6 pregnant animals. Each mice was given daily intraperitoneal injection of 0.7 mg/100 gms B.W. of aluminium sulphate for periods given in Table 1 and sacrificed on day 20 of gestation.

Recovery, fixation and preservation of embryos

On day 20 of pregnancy the animals were sacrificed and the two horns of uteri containing the embryos were dissected out. The embryos along

with uteri were then fixed in buffered formalin. Forty eight hours after fixation, the crown-rump (C-R) length of the embryos was measured by using a millimeter scale.

Table 1: Experimental design.

Control			*Experimental*		
Group	*Dose*	*Period**	*Group*	*Dose*	*Period**
A	0.25 ml of dist. water	1-6	A1	0.25 ml of $Al_2(SO_4)_3$ solution	1-6
B	-do-	7-12	B1	-do-	7-12
C	-do-	13-18	C1	-do-	13-18
D	-do-	1-12	D1	-do-	1-12
E	-do-	7-18	E1	-do-	7-18
F	-do-	1-18	F1	-do-	1-18

*Days of gestation.

Statistical analysis

The statistical analysis of results regarding the crown rump length of embryos recovered was done using independent sample t-test and analysis of variance. A p-value ≤ 0.05 was considered significant for all analyses.

RESULTS

Morphological and morphometric observations

Control group

Twenty day old embryos of different groups recovered were all well developed. Their morphological appearance were almost similar to that described in the classical description of mouse embryogenesis[18]. All these embryos were quite similar in general appearance and in anatomical details with minor variations in crown rump length and weight. Average crown rump length of control embryos was 25.5 mm and their weight turned out to be 1.061 mg.

All these embryos had a fully developed semicurved body which was distinctly divided into head and neck, trunk and tail region.

Experimental embryos

Following administration of Aluminium sulphate for various periods, described already, some interesting observations were made. It was noted that this compound was toxic to the mothers as well, when used for long time e.g. mothers of F1 group. Out of six mothers only 3 survived and all of them were listless, weak and exhausted on day 20 of pregnancy. Skin changes were also observed in mothers of group D1, E1 and F1. The changes were most marked in group F1 which showed typical scaly dermatitis specially of dorsal skin.

The weight gain of the mothers of experimental groups was also very slow as compared to control groups. When administered for small periods compound did not produce any sign and symptom of toxicity in the mothers, although the embryos recovered from these mothers showed dwarfism, reduced body weight and retarded development.

0.7 mg/day of $Al_2(SO_4)_3$ when used only for one week i.e. in A1, B1 and C1, did not produce any marked toxicity in mothers. But mothers did show increased respiratory activity and decreased locomotor activity during that particular period f dose administration. All those toxic symptoms disappeared within 24 hours of stoppage of drug. All the 6 mothers in each group survived till day 20 of pregnancy.

A similar dose of $Al_2(SO_4)_3$ when used for longer periods i.e. in groups D1 and E1, did show toxicity regarding physical activities of mothers. In group E1 five out of six mothers survived till day 20 of pregnancy. Still longer periods of dose administration as done in group F1 showed severe toxic symptoms in all the mothers and only three out of six were able to survive from these acute toxic effects.

Dissecting microscope study of embryos recovered from the surviving mothers showed that they were negatively affected by this heavy metal only when it was used for prolonged periods during pregnancy. Crown rump length was significantly decreased only in groups D1, E1 and F1. The dwarfism was more marked as the duration was increased. The body of the treated embryos, similar to the control groups embryos, was distinguishable into head and neck, trunk and tail regions in all the groups. But the appearance of group E1, and F1 embryos were more towards prematurely or retarded development.

The statistical analysis of results obtained regarding the C.R. length of embryos recovered is given in Table 2.

Table 2: Statistical analysis of crown rump length.

I) T-test

Groups	Mean		Mean difference from control	Standard error	Computed value of t	P-value (2-tailed)
	Control (mm)	Experimental (mm)				
A	25.95	25.05	0.90	0.434	2.08	0.06
B	25.65	24.93	0.72	0.367	1.95	0.08
C	24.53	24.03	0.50	0.283	1.77	0.11
D	25.30	22.07	3.23	0.370	8.73	0.000
E	25.15	22.02	3.13	0.519	6.06	0.000
F	26.03	15.03	11.00	0.815	13.50	0.000

II) Analysis of variance

Control to Experimental groups		Sum of squares	D.F	Mean square	F	P-value (2-tailed)
A-A1	Between groups	2.430	1	2.430	4.316	0.064
	Within groups	5.630	10	0.563		
B-B1	Between groups	1.541	1	1.541	3.806	0.080
	Within groups	4.048	10	0.405		
C-C1	Between groups	0.750	1	0.750	3.116	0.108
	Within groups	2.407	10	0.241		
D-D1	Between groups	31.363	1	31.36	76.248	0.000
	Within groups	4.113	10	0.411		
E-E1	Between groups	71.773	1	71.77	97.533	0.000
	Within groups	6.623	9	0.736		
F-F1	Between groups	242.00	1	242.00	182.15	0.000
	Within groups	9.300	7	1.329		

DISCUSSION

The results of present research work indicate that the aluminium compounds are not only teratogenic and embryotoxic but detrimental for the pregnant mothers as well. The cholinergic signs which appeared in mothers of group D1, E1 and F1, indicated toxicity of aluminium sulphate. Recovery of mothers from these toxic effects was 100% in group D1, 90% in group E1, and only 50% in group F1.

In group A1 neither any gross abnormality nor any noticeable effect on C-R length was found. These results are similar to those of Wide[19] who studied the effects of short term exposure of five industrial metals on the embryonic fetal development of the mouse. He found none of the compound including aluminium given before implantation or in early periods of pregnancy caused any fetal malformations. In the present study along with the short term exposure, the prolonged exposure effects have also been observed. The short

term exposure has produced the same results as of aforesaid researchers but prolonged exposure to aluminium compounds has been proved to affect the C-R length.

While experimenting on chick embryos Gilani and Chatzinoff[20] studied the teratogenic potentials of aluminium. They found aluminium to be embryo-lethal and had some tendency to reduce body size and weight in chick embryos. Similarly in 1988 Petermain[21] used a daily dose of 180, 380, 720 mg/kg of aluminium nitrate from the sixth through the fourteenth day of gestation. Fetal examination were performed on day 20 of pregnancy. In this study the results of present research work are similar to those of aforesaid workers as far as body weight and size is concerned. None of these researchers have correlated the results of exposure for long and short periods, which has been studied in present project.

In group B1 not only the crown-rump length but also the renal development was found to be affected. In group C1 the effect of crown-rump length and fetal body weight were though present, but after statistical analysis it proved to be statistically insignificant, McCormack[17] concluded that aluminium was not teratogenic in rats. In his experiment the rats were free and allowed to ingest aluminium on their own. No serum study was done to find out the concentration of aluminium. According to Lione[7] the amount of aluminium ingestion depends upon animal's likes and dislikes, the absorption of aluminium depends upon the acidity of stomach at that particular time and the effects at tissue level depend upon its clearance from the kidneys. So in an attempt to maintain constant serum levels the aluminium was injected intraperitoneal to by pass the gastrointestinal tract.

CONCLUSION

This preliminary animal study shows that there may be intra-uterine growth retardation with long term use of aluminium containing compounds by pregnant mothers. This could have important implications while prescribing the drug to pregnant mothers.

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