Developmental Toxicity of Aluminum from High Doses of AlCl3 in Mice

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ABSTRACT

Aluminum (Al) has a significant toxic potential for humans. Although knowledge of Al toxicity has markedly improved in recent years, there is relatively little information regarding its embryotoxicity and teratogenic potential. The purpose of this study was to assess the effects of short-term exposure of aluminum chloride (AlCl3) on the external abnormality of the fetuses of pregnant mice.

Mature Naval medical research institute (NMRI) mice (24 to 33 g) were used in this study. Day 0 of gestation was defined as the day in which the vaginal plug was observed. Plug-positive mice were randomly divided in to six groups, each composed of 12 mice. To the first, second, and third groups, 150 mg/kg of AlCl3 was injected intraperitoneally on the tenth, eleventh, and twelfth days of gestation, respectively. Normal saline (0.3 mL) was injected in the mice in the control groups on the same days of gestation. Mice were killed on day 18 of gestation. Live fetuses were weighed and examined for external abnormalities.

The fetal body weight was significantly reduced in all Al-treated groups ($P<0.05$). The external anomalies on the fetuses in groups 1, 2, and 3 were 28.8%, 20.3%, and 13.0%, respectively, with significant differences compared with the control groups ($P<0.001$). It is concluded that a single dose of Al administered to a pregnant mouse can cause fetal death, decreased body weight, and potential external anomalies in the fetus.

INTRODUCTION

Aluminum (Al) is an important element with known toxicity in the human body, mainly in the central nervous system.[1] Its toxic effects have been investigated for many years. Al is known as a neurotoxin that can cause certain diseases such as Alzheimer disease, dialysis dementia, Parkinsonism, and amyotropic lateral sclerosis.[2-5] In addition to its neurotoxicity, Al affects other body structures like the skeletal system,[6] brain tissue, and blood cells.[6-8] In spite of the known toxicity of Al, until recently, there was little concern about toxic consequences of Al ingestion because it was assumed that Al was not orally bioavailable. However, in recent years, it

has been shown that although the gastrointestinal tract normally represents a major barrier to Al absorption, under some circumstances this barrier can be breached. Consequently, individuals ingesting large amounts of Al compounds do absorb a definite amount of Al.[1,9] Environmental pollution with the different aluminum-containing compounds, specially those in industrial waste water, exposes people to higher than normal levels of Al.[10] Particulate matters distributed by cement-producing factories contain high amount of Al, and populations residing in the vicinity are exposed to the pollution.[11]

Although some studies were undertaken to examine the toxic effects of Al-containing substances on matured animal models, further studies must be performed to investigate the teratogenic effects of Al on mammal embryos.[1]

As Al is widely used in antacid drugs, which are frequently used by pregnant women,[1,12] as well as in food additives, toothpaste, and some cosmetics, it is of great importance to increase the knowledge about the toxic effects of Al on the fetus. It should be kept in mind that many toxic compounds that may be tolerable in certain concentrations in adults are harmful for fetuses.[13] Several reports indicate the toxic effects of different concentrations of Al on an embryo/fetus.[14-16] However, controversy on its toxic effects remains to be fully resolved as some studies fail to show embryotoxic effects for Al.[12,17] The purpose of this study was to assess the effect of a single high dose of Al to pregnant mice on the external structural development of their offspring.

MATERIALS AND METHODS

Animals

Female and male Naval medical research institute (NMRI) mice were obtained from Iranian Pasteur Institute. Animals were housed in plastic cages in a constant light-dark cycle (12:12 hr) at temperature of 23°C ± 2°C, and were fed adequately.

Mature female mice (24 to 33 g) were mated with males (2:1) overnight and examined the following morning for copulatory plugs. Day 0 was defined as the day in which the vaginal plug was found.

Chemicals and Treatments

Aluminum chloride (AlCl3) was obtained from Merck (Darmstadt, Germany), and was administered in aqueous solution (using normal saline). Plug-positive female mice were randomly divided into six groups (12 in each group), and they received the following treatments. To the first three groups, a single dose of 150 mg/kg AlCl3 was administered intraperitoneally on days 10, 11, and 12 of gestation, respectively. Groups 4, 5, and 6 were the controls for the first three groups, respectively, and received a 0.3-mL injection of normal saline on the same day.

Mice were killed on day 18 of gestation by neck dislocation. Fetuses were removed, weighed, and their crown-lump (CR) length measured accurately. The external macroscopic features were studied stereomicroscopically.

Statistical Analysis

For comparison of mean weight and CR length of fetuses, ANOVA, and Tukey tests were used. $P<0.05$ was considered the level of significance.

RESULTS

Table 1 summarizes the body weight and CR length of the fetuses in the different groups. The body weights of the fetuses were 0.283, 0.290, and 0.303 g in the experimental groups and 0.321, 0.323, and 0.325 g in the control groups, respectively. The body weight was significantly reduced in all Al-treated groups ($P<0.05$).

Table 1. Mean Weight and Crown-Rump Length of the Fetus in the Experimental and Control Groups.

	Weight (g)*	CR Length (mm)
Group	**Mean ± SD**	**Mean ± SD**
Day 10 with Al exposure	0.283 ± 0.03	12.9 ± 0.7
Day 10 control	0.321 ± 0.04	13/2 ± 0.5
Day 11 with Al exposure	0.290 ± 0.03	13.2 ± 0.5
Day 11 control	0.323 ± 0.02	0.321 ± 0.04
Day 12 with Al exposure	0.303 ± 0.02	13.2 ± 0.4
Day 12 control	0.325 ± 0.03	13.1 ± 0.7

*Difference between experimental and the relevant control group was significant, $P<0.05$.
Al = aluminum
SD = standard deviation

Table 2. Fetal Anomalies and Their Frequencies in the Mice Injected With AlCl3 on Days 10, 11, and 12 of Gestation

Types of Fetal Anomaly	Day 10 (N=73) n (%)	Day 11 (N=74) n (%)	Day 12 (N=69) n (%)
Syndactyly	10 (13.7)	4 (5.4)	5 (7.2)
Other anomalies of the limbs	1 (1.4)	2 (2.7)	1 (1.4)
Anencephaly	3 (4.1)	2 (2.7)	1 (1.4)
C form fetuses (excessive curve in spinal column)	3 (4.1)	2 (2.7)	0 (0)
Subcutaneous bleeding	4 (5.5)	5 (6.8)	2 (2.9)
Total	21 (28.8)*	15 (20.3)	9(13.0)

* Significant compared to day 12 group ($P<0.05$).

However, there were no significant differences among groups in the CR length. All types of external major malformations and their frequency in the different groups are summarized in Table 2. Frequency of gross external malformations in the Al-treated groups were significantly higher ($P<0.001$). These ratios for the fetuses of the experimental groups of days 10, 11, and 12 were 28.8%, 20.3%, and 13.0%, and 3.4%, 4.3%, and 4.1% for the control groups, respectively.

In the Al-treated groups, the resorption rates/fetus were 25.5%, 21.2%, and 23.3%, respectively, while no resorption in the control groups was noted. External skeletal deformities were seen most commonly in the lower and upper limbs, spinal column, and skull in the fetuses of the experimental groups. In addition, the anomaly rate increases considerably with fetuses that were exposed earlier in gestation. However, only the difference between groups 1 (day 10 of gestation) and 3 (day 12 of gestation) was significant ($P<0.01$).

DISCUSSION

Findings of this study indicate that AlCl3 has severe toxic effects on the mouse embryo/fetus, which leads to significant increase in resorption rate, decreased body weight, and major anomalies. In previous studies using different Al-containing components, the reported results show some controversy. While Golub et al showed that injection

of Al lactate (15 to 40 mg/kg) in pregnant mice on different days of the gestation period had no effect on the weight of fetuses and did not increase anomalies,[17] Paternain et al reported that Al nitrate (13 to 62 mg/kg) caused weight loss and increased anomalies of external structures and the skeletal system in rat fetuses.[15]

In another study that administered 100 to 200 mg/kg of AlCl3 in rats on days 14 to 18 of gestation caused growth retardation, increase of atrophy and fetal death, as well as skeletal anomalies.[18] Colomina et al studied the effects of AlCl3 (75 mg/kg) on days 6 to 15 of gestation on the embryos/fetuses of mice and noticed low body weight, but the number of external skeletal anomalies did not increase.[16] It seems the above-mentioned differences reflect a difference in the source and dosage of Al, route and frequency of administration, and time of Al exposure. The results of this study showed that 150 mg/kg of AlCl3, has high toxicity for mouse fetuses, to such an extend that injection of a single dose on either day 10, 11, or 12 of gestation causes significant increase on the incidence of external skeletal anomalies.

Aluminum ion (Al3+) 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[13,19] and inhibits embryonic cell proliferation and protein synthesis. This mechanism can explain the toxic effects of Al on the embryo and fetus. There are also other cellular mechanisms by which Al is thought to 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.[12]

On the basis of these findings, it is concluded that high doses of AlCl3 show toxic effects on mouse embryos/fetuses, and even a short exposure to it can lead to fetal death, decreased body weight, and skeletal anomalies.

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