EFFECT OF INTRAPERITONEAL ADMINISTRATION OF ZINC IN C57/6J MICE

B H Bay, K H Sit

ABSTRACT

Intraperitoneal administration of zinc chloride to C57/6J mice in vivo at 28 μg/g body weight was more lethal to the male mice and induced growth retardation in the male and female survivors. In the group of experimental survivors, the weight of the lungs were significantly decreased compared with the control animals. The inherent dangers of zinc excess is highlighted, especially with regard to the possibility of cumulative zinc toxicity.

Keywords: Zinc chloride, in vivo, zinc toxicity, lethal.

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INTRODUCTION

Zinc, an ubiquitous trace biological element, is involved in more than 235 metalloenzymes[1] including thymidine kinase, RNA polymerase and ribonuclease, which in turn play a crucial role in the replication and transcription of DNA during cell division[2-4]. Considered one of the least toxic metals in the environment[5], much focus has been on zinc deficiency and the resultant effects such as growth retardation and failure to thrive in infants[6,7] and impairment of the immune system[8,9]. The protective role of zinc against radical tissue damage which may contribute to chronic health problems including cancer, has been the subject of great interest[4].

However, the potential consequences of excessive zinc intake such as sideroblastic anaemia and bone marrow depression[10,11] must be realised. It has been previously shown in Swiss albino mice that intraperitoneal administration of zinc chloride, induced chromosomal aberrations of the bone-marrow cells and sperm head abnormalities at doses varying from 1/15 to 1/10 LD_{50} in chronic treatment and 1/4 to 1/2 the LD_{50} for acute treatment (mice sacrificed after 24 hours)[5]. In this study, the toxic effects of intraperitoneal zinc chloride in C57/6J mice are evaluated.

MATERIALS AND METHODS

Chemical

Zinc chloride ($ZnCl_2$) (Analar grade, Merck, Germany) was dissolved in Type I reagent water (Miili-Q system, USA). Stock solution of $ZnCl_2$ was 25 mM strength.

Animals

For the experiment, 5-6 weeks age laboratory-bred C57/6J black mice litter-mates with an average weight of 12g for the females and 15g for the males, were maintained on a standard balanced diet.

Experimental Protocol

Eight male and eight female mice were designated as controls and intraperitoneal(ip) injections of 0.1 ml normal saline (140 mM NaCl) were administered five times/week.

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Another eight male and eight female mice were designated as experimental animals and ip injections of $ZnCl_2$ at a dosage of 28 μg/g body weight (equivalent to LD_{50} dose of female C57/6J mice obtained previously) were give five times/week.

At the end of 25 days the female mice were sacrificed and weighed. The lungs were then dissected out, fixed in Bouin's solution and dehydrated in 70% alcohol before weighing. Blood was taken from the anaesthesized female experimental and control animals via the intracardiac route.

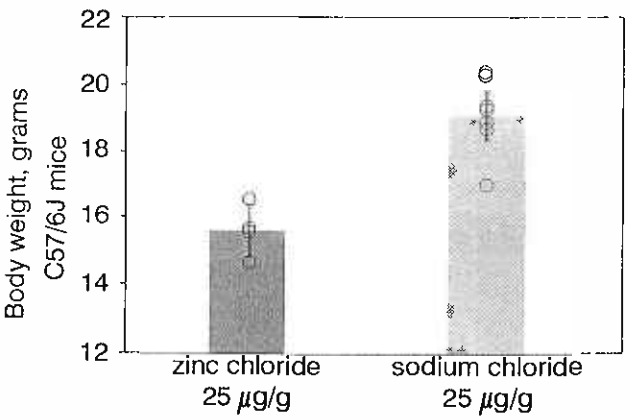
RESULTS

Of the eight male experimental mice, one survived while four female experimental mice were still alive on the 25th day (Table I). The 87.5% mortality rate in the male experimental mice was much higher than the corresponding 50% mortality rate in the female animals. With regard to the total body weight between the female experimental and corresponding control animals at the end of the experiment, Fig 1 shows that the

Table I – Lethal effect of intraperitoneal zinc on male and female C57/6J mice

C57/6J mice	Female	Male
Experimental	8	8
No. of Deaths	4	7

Fig 1 – Total body weight of female zinc treated mice versus corresponding controls administered intraperitoneal saline. Error bar = 2 Std. Error.



difference between the groups are significant to the extent of 95% confidence limits as indicated by the non-overlapping twice standard error bars. A similar result was found in the lung weights (Fig 2). The survivors in the experimental group were observed to have patchy loss of hair as well. The haematological values of the female control and experimental animals (Table II) were within normal limits[12].

DISCUSSION

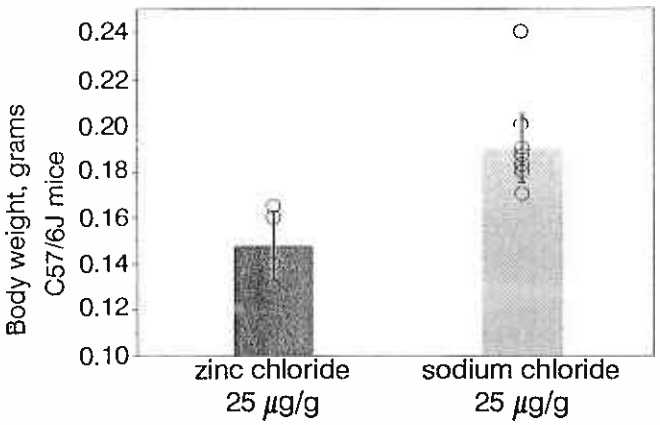
Dreosti et al[13] have shown that feeding zinc deficient diets (>10 ppm) to pregnant C57/6J mice, would result in the total loss of offspring and severe teratogenesis. On the other hand, we show here that loading the same species of mice with zinc could lead either to death or induce marked weight loss with associated patchy alopecia. Similarly there was also a loss of total lung mass. The lung is a non-reticuloendothelial organ, easily accessible, accurately dissectible and uninvolved in haemoglobin synthesis. The weight loss in the lungs therefore appears to support the possibility of a general toxic effect. The dosage used in this experiment apparently did not cause anaemia.

This in vivo study demonstrates clearly that excessive amounts of zinc could be toxic. The inherent dangers of self-supplementation with large doses of zinc should therefore always be borne in mind[14]. Since zinc is increasingly used in total parenteral nutrition[15], the possibility of zinc toxicity must be considered if the patient develops symptoms such as nausea, vomitting, lethargy or anaemia from zinc induced copper deficiency[14,16] especially with regard to long term cumulation of zinc[5]. A growing appreciation of the potential adverse effects of zinc in humans and other species has led to the timely call for enforcing an upper limit for zinc in infant formulae[17].

Table II – Comparison of haematological parameters between zinc treated and control female mice (only the values of one animal in each group is reported here).

	Control	Experimental
Haemoglobin (g/dl)	16.1	15.7
Hematocrit (%)	51.3	49.4
Mean Corpuscular Volume (fl)	50.9	47.0
Mean Corpuscular Haemoglobin (pg)	15.9	15.0
Mean Corpuscular Haemoglobin Content (g/dl)	31.3	31.8

Fig 2 – Lung weight of female zinc treated mice versus control animals. Conditions were similar to those in Fig 1. Error bar = 2 Std. Error.



It is also interesting to note that while four of the female experimental animals survived, only one male experimental animal survived. This serves to illustrate the fact that the sex of the mice play a major role in the tolerance of zinc perhaps because of the different genetic constitution. Looking at this with a broader perspective, the calculation of toxic doses of drugs based on body weight alone, without taking into account the constitutional factors and cumulative effects, may therefore not be as accurate as reflected.

In in vitro studies, a much higher level of zinc concentration has been reported to be cytotoxic[18]. The in vivo dosage in our experiment was only 0.2 µM (assuming that two-thirds of the body weight of the mouse is extracellular fluid). Since a much higher in vitro dosage (40 µM or 200 times our in vivo dosage) has been reported to cause an increase in EGF-stimulated DNA synthesis[19], it would appear that there are other mechanisms apart from cell proliferation, causing the effects observed here. Zinc is known to be a specific inhibitor of sulphotransferase in detoxication mechanisms[20]. One possibility of zinc toxicity could be due to the abolition of this metabolic pathway. For example, we have found that patchy hair loss in zinc treated mice is a cytodifferentiation effect whereby coarse hair is replaced by fine hair[21].

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