

EFFECT OF INTRAPERITONEAL ZINC ON THE HAEMATOLOGICAL PROFILES OF C57/6J MICE

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ABSTRACT

Zinc is included in a number of medications and haematotoxic effects due to zinc excess have been reported. In this study, the haematological profiles (haematocrit, haemoglobin, total white blood cell count, differential white blood cell count and platelets) of mice treated with zinc were evaluated. Intraperitoneal zinc chloride was administered to C57/6J mice in varying dosages from 1.4-14 µg/g body weight, four times a week for a period of three weeks. Zinc chloride had no effect on the haematological profiles of these mice since the blood cell counts of treated mice were not significantly different from the controls ($p < 0.05$), with the exception that at half LD_{50} of zinc chloride (14 µg/g body weight) a reactive thrombocytosis resulted. (The platelet counts between the control and experimental group of mice were significantly different, exceeding 95% confidence limits; $p = 0.016$). We postulate that the specific effect on platelets was due to zinc being a potent inhibitor of phenol sulfotransferase (PST), an enzyme which is involved in many metabolic pathways. Platelets are a rich source of PST and the thrombocytosis observed was probably a compensatory mechanism to raise the levels of PST in the body.

Keywords: zinc chloride, haematocrit, white cell count, platelets, thrombocytosis

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INTRODUCTION

Zinc is contained in dental amalgams, vitamin supplements, fungicides and administered in total parenteral nutrition⁽¹⁻³⁾. In recent years, adverse effects of zinc excess are increasingly reported^(4,5). Haematotoxic effects of zinc excess which have been reported include sideroblastic anaemia and depression of bone marrow^(6,7). There have also been reports of an increase in chromosomal aberrations of bone marrow cells in Swiss albino mice which were subjected to intraperitoneal zinc treatment⁽⁸⁾.

In view of the above reports, the aim of the present study was to evaluate whether the administration of zinc in vivo affected the blood profiles of the experimental animals. In this study, intraperitoneal (i.p.) zinc chloride was administered to C57/6J mice for three weeks and the haematological profiles viz haemoglobin, haematocrit, total white cell count, differential white cell count and platelets were analysed.

METHODS

Fifteen female mice (C57/6J strain, 5-6 weeks old) with an average weight of 12 g were maintained on a standard balance diet as previously described⁽⁹⁾. The mice were divided into five groups with each group consisting of three animals. The group designated as controls received i.p. injection of distilled water (0.1 mL four times a week for three weeks). The other four groups received zinc chloride i.p. at varying dosages of 1.4 µg to 14 µg/g body weight administered in the same volume and frequency as the control animals. The maximum

dose of 14 µg/g bodyweight is equivalent to half LD_{50} . The LD_{50} dose of female C57/6J mice had been previously established as 28 µg/g body weight⁽⁹⁾. Zinc chloride (Analar grade, Merck, Germany) was dissolved in Type I reagent water (Milli-Q system, USA) to give a 25 mM strength stock solution. On the 22nd day, blood samples were obtained from i.p. chloral hydrate-anaesthetised mice by intracardiac puncture. Haematological profiles were analysed automatically in a HI Technicon blood analyser.

Results are expressed as mean and sem. The statistical differences were determined using 1-way ANOVA (Analysis of Variance) and student's *t*-test. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

As shown in Table I, the haemoglobin values of the zinc treated mice were not significantly different from the control animals ($p = 0.40$). The haemoglobin values of all the animals in the five groups were within normal limits⁽⁹⁾. Similarly, there was no significant difference in the haematocrit values of both control and treated mice as evidenced by the overlapping twice standard error bars in Fig 1. Neither were there any significant differences in the total white blood cell count (Table I) ($p = 0.93$) nor in the differential white cell counts (Table II) between treated and untreated animals. However as shown in Fig 1, there was a significant difference between the platelet counts of the control group and the mice which were administered zinc chloride at a dosage of 14 µg/g body weight (half LD_{50}) to the extent of 95% confidence limits as represented by the non-overlapping twice standard

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Table I - Effect of zinc on the haemoglobin and total white blood cell count in female C57/6J mice

Zinc dosage (µg/g body weight)	Haemoglobin (g%) (mean ± sem, n=3)	Total White (per µl) (mean ± sem, n=3)
0	13.8 ± 0.3	1537 ± 267
1.4	12.6 ± 0.4	1806 ± 575
2.8	13.8 ± 1.0	1646 ± 386
5.6	13.7 ± 0.6	2166 ± 1060
14	13.9 ± 0.4	1923 ± 736

Fig 1 - Effect of intraperitoneal zinc chloride on the haematocrit and platelet counts in female C57/6J mice.

Haematocrit is expressed as percentage (linear regression plot). For platelet counts, the scale on the y-axis represents number of platelets $\times 10^3$ (2nd degree polynomial regression plot). The error bars represent twice standard sem (95% confidence limits). r is Pearson's correlation coefficient.

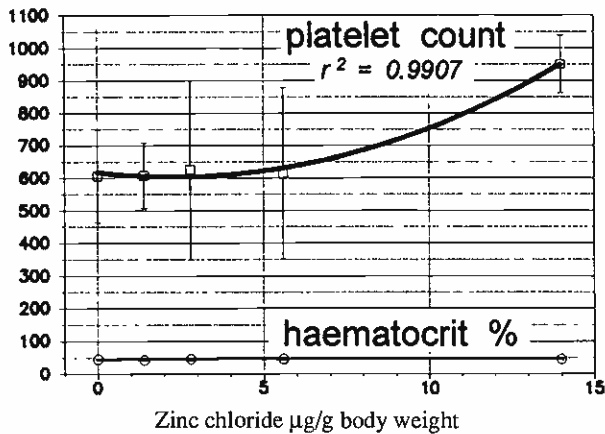


Table II - Effect of zinc on the differential white blood cell count in femal C57/6J mice

Zinc dosage ($\mu\text{g/g}$ body weight)	Differential Count (%) (mean \pm sem, n=3)				
	N	L	M	E	B
0	6.3 \pm 3.3	82.0 \pm 3.2	10.7 \pm 2.8	0.06 \pm 0.04	0.9 \pm 0.2
1.4	4.9 \pm 1.9	81.3 \pm 2.8	12.6 \pm 2.8	0.2 \pm 0.1	0.6 \pm 0.2
2.8	5.4 \pm 2.8	79.2 \pm 5.7	12.0 \pm 2.6	0.3 \pm 0.1	1.3 \pm 0.6
5.6	6.9 \pm 0.8	77.0 \pm 8.5	9.5 \pm 1.6	0.1 \pm 0.03	0.3 \pm 0.1
14	4.9 \pm 0.5	76.9 \pm 3.3	14.5 \pm 3.9	0.1 \pm 0.07	0.4 \pm 0.2

N=neutrophils, L=lymphocytes, M=monocytes, E=eosinophils, B=basophils

error bars. The platelet counts in these two groups were also statistically significant ($p=0.016$) when analysed with the student's t -test.

DISCUSSION

Contrary to previous reports^(6,7), the administration of intraperitoneal zinc did not decrease the haemoglobin nor haematocrit levels in zinc treated mice. The total number of white blood cells including the differential counts were also unaffected although the possibility of impaired leucocyte function cannot be excluded⁽¹⁰⁾.

The observed 50% rise in platelets could be due to inhibition of phenolsulfotransferase (PST) by zinc. PST is a cytosolic enzyme that catalyses the conjugation of phenolic drugs, catecholamines, neurotransmitter substances and xenobiotic compounds^(11,12). Zinc, a known strong inhibitor of PST, is reported to reduce the activity of PST to only 3-5% of its initial activity⁽¹³⁾ and platelets are an important and rich source of this enzyme^(14,15). The increase in platelets could therefore have been a compensatory mechanism to raise PST level in the body since this enzyme is essential in many

pathways for detoxication and metabolic inactivation. At lower levels of zinc dosages, there was no reactive thrombocytosis probably because there was still sufficient PST activity.

CONCLUSION

In this study, we have shown that although the blood profiles of C57/6J mice were essentially unaffected by zinc treatment, there was a specific effect on platelets when given at a high dose. An increase in platelets can be deleterious to health especially in elderly patients who have cerebrovascular or coronary insufficiency. This is because platelets are known to participate in atherogenesis, form the nidus of thrombus and release vasoactive substances that cause vasoconstriction⁽¹⁶⁾. Moreover, zinc at high concentrations can itself induce platelet aggregation and also potentiate adenosine diphosphate induced platelet aggregation via protein kinase C^(17,18). In view of the widespread use of zinc, the call for concern about the potential for zinc excess among the elderly, and with it the possible adverse effects, is therefore timely^(19,20).

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