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

**B H Bay, G Singh, K H Sit**

*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*

**SINGAPORE MED J 1995; Vol 36: 271-272**

## 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[5]. 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[5]. 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 H1 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

**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 |

**Department of Anatomy**
**National University of Singapore**
**Kent Ridge**
**Singapore 0511**

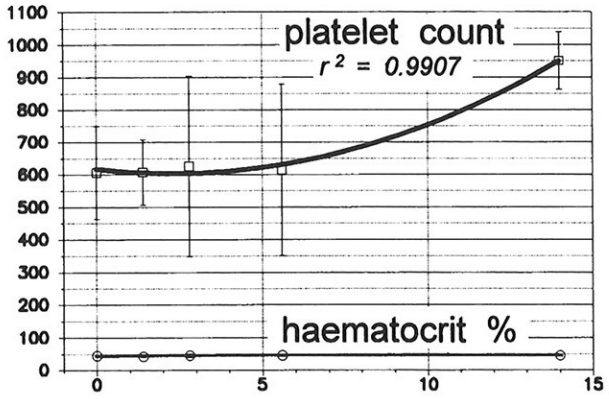
B H Bay, MBBS, PhD
Lecturer

G Singh, MBBS, FRACS
Senior Lecturer

KH Sit, MBBS, MD, PhD (Lond)
Professor

**Correspondence to :** Dr B H Bay

**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 x $10^3$ (2nd degree polynomial regression plot). The error bars represent twice standard sem (95% confidence limits). r is Pearson's correlation coefficient.**




Zinc chloride µg/g body weight

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

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


## ACKNOWLEDGEMENTS

The authors would like to thank Mr. Tajuddin B.M. Ali (Operations Theatre, Department of Anatomy) and Ms. L.P. Tam (Clinical Laboratory Medicine, National University Hospital) for their technical assistance.


## REFERENCES


1. Phillips GD. Zinc in total parenteral nutrition. In: Prasad AS, Dreosti IE, Hetzel BS. eds. Clinical applications of recent advances in zinc metabolism. New York: Alan R Liss Inc, 1982:169-80.
2. Kaga M, Seale NS, Hanawa T, Ferracane JL, Waite DE, Okabe T. Cytotoxicity of amalgam alloys, and their elements and phases. Dent Materials 1991; 7:69-72.
3. Bay BH, Sit KH. Coarse to fine hair conversion induced by zinc in C57/6J mice. Tohoku J Exp Med 1992; 168:63-6.
4. Fosmire GB. Zinc toxicity. Am J Clin Nutr 1990; 51: 225-7.
5. Bay BH, Sit KH. Effect of intraperitoneal administration of zinc in C57/6J mice. Singapore Med J 1993; 34:58-9.
6. Broun RE, Greist A, Tricot G, Hoffman R. Excessive zinc ingestion. JAMA 1990; 264:1441-3.
7. Forman WB, Sheehan D, Cappeli S, Coffman B. Zinc abuse: an unsuspected case of sideroblastic anemia. Western J Med 1990; 152:190-2.
8. Gupta T, Taluder G, Sharma A. Cytotoxicity of zinc chloride in mice in vivo. Biol Trace Elem Res 1991; 30:95-101.
9. Hardy J. Haematology of rats and mice. In: Cotchin E, Roe FJC. eds. Pathology of laboratory rats and mice. Oxford and Edinburgh: Blackwell Scientific Publications, 1967:501-36.
10. Prasad AS. History of zinc in human nutrition. In: Prasad AS, Dreosti IE, Hetzel BS. eds. New York : Alan R Liss Inc., 1982:1-17.
11. Khoo BY, Sit KH, Wong KP. The human platelet as an independent unit for sulfate conjugation. Life Sci 1988;42:1161-71.
12. Otterness DM, Powers SP, Miller LJ, Weinstilboum RM. Human liver thermostable phenol sulfotransferase: photoaffinity labelling with 2-iodo-4-azidophenol. Mol Pharmacol 1989;36:856-65.
13. Baranczyk-Kuzma A, Szymcyzk T. Extrahepatic sulfation of phenols. Biochem Pharmacol 1987; 118:500-9.
14. Wong KP, Khoo BY, Tan TMC, Sit KH. Phenolsulfotransferase (PST) and PAPS in catecholamine metabolism in human tissues: an overview. J Neurol Transm (Suppl) 1990;29:163-71.
15. Anderson RJ, Garcia MJ, Liebentritt DK, Kay DH. Localization of human blood phenol sulfotransferase activities: novel detection of the thermostable enzyme in granulocytes. J Clin Lab Med 1991; 118:500-9.
16. Braunwald E. Impact of cell and molecular biology on cardiovascular disease. In: Wilson JD, Braunwald E, Isselbacher K, Petersdorf RG, Martin JB, Fauci AS, Root RK. eds. Harrison's Principles of Internal Medicine. New York: McGraw Hill Inc 1991: 835-41.
17. Heyns A, Eldor A, Yarom R, Marx G. Zinc induced platelet aggregation is mediated by the fibrinogen receptor and is not accompanied by release or by thromboxane synthesis. Blood 1985; 66: 213-9.
18. Kowalska MA, Juliano D, Trybulec M, Lu W, Niewiarowski S. Zinc ions potentiate adenosine diphosphate-induced platelet aggregation by activation of protein kinase C. J Lab Clin Med 1994; 123: 102-9.
19. Frambach DA, Bendel RE. Zinc supplementation and anaemia. JAMA 1991; 265:869.
20. Kalfakakou WP, Evangelou AM, Benveniste J, Arnoux B. The effects of zinc on guinea pig isolated heart preparations. Biol Trace Elem Res 1993; 38:289-300.