Pharmacokinetics of Zinc in Pre-Diabetes: A Pilot Study

Abstract

Background: Zinc is an essential trace element that plays a vital role as a co-factor in enzyme action. It is also important in insulin action and carbohydrate metabolism. Understanding Zinc metabolism in the pre-diabetic state could help to define the role of Zinc in the pathogenesis of diabetes mellitus. The present study aims to investigate the pharmacokinetic parameters of Zinc in pre-diabetes using the oral Zinc tolerance test.

Methods: The present study was conducted as a pharmacokinetic sub-study of a randomized controlled trial evaluating the effects of Zinc supplementation in pre-diabetes. Initially a baseline blood sample was taken (0hrs) after 10 hours of overnight fasting. The Zinc 20mg capsule was given to the patient to be taken orally after obtaining this baseline blood sample (0hrs). Subsequent blood samples were taken at 30 min, 1h, 2h, 3h and 6h. A 24 hour sample was taken in the morning of the following day. Subjects were given standard meals during the period of the study. Serum Zinc was analyzed by colorimetric method. Pharmacokinetic parameters were calculated using the non-compartment extra-vascular model applied for the Zinc plasma disposition curves.

Results: Sample size was 10, of which four patients were males. Mean age (±SD) was 52.6±9.6 years. The serum Zinc concentration of all study participants with pre-diabetes prior to the commencement of Zinc supplementation in the clinical trial were below the normal range. The mean serum Zinc concentration (±SD) at baseline (0 hours) was 10.63±3.0 µmol/l, which was higher than the mean pre-supplementation Zinc concentration (9.06±1.93 µmol/l) (p=0.10). The maximal concentration of Zinc (±SD) achieved in the blood (Cmax) was 23.56±4.46 µmol/l and Tmax was 2 hours. Subsequently the Zinc concentration gradually decreased, however at 24 hours an increase in the mean Zinc concentration was observed. The elimination half life (T½) was 4.91 hours.

Conclusion: Our results show the presence of hypozincaemia in those with pre-diabetes, which was improved with Zinc supplementation. Zinc absorption was normal in the study population, however elimination half life was prolonged. Furthermore, there is possible impairment in entero-hepatic re-circulation observed in this population with pre-diabetes.

Keywords: Zinc supplementation; Zinc pharmacokinetics; Pre diabetes

Research Article

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Abbreviations: AUC: Area Under the Curve; BMI: Body Mass Index; GCP: Good Clinical Practice; IFG: Impaired Fasting Glucose; IGT: Impaired Glucose Tolerance; ITI: Industrial Technology Institute; SD: Standard Deviation; WHO: World Health Organization

Introduction

Zinc is an essential trace element that plays a vital role as a co-factor in enzyme action, cell membrane stabilization, gene expression and cell signaling [1]. It is also important in insulin action and carbohydrate metabolism [2]. Zinc is involved in the physiology of insulin at several stages; it is found in the insulin secretory granules and is known to participate in the insulin synthesis, stabilization of pro-insulin, insulin secretion, insulin sensitivity, and insulin degradation [3,4]. Zinc could also play a role in the pathogenesis of diabetes. Studies have shown that diabetes is accompanied by hypozincemia and hyperzincuria [5,6]. Zinc absorption is also know to be altered in patients with diabetes [7]. The altered Zinc absorption and hyperzincuria identified in patients with diabetes is an indication of either the fact that Zinc metabolism is altered as a result of diabetes or the altered Zinc metabolism plays a role in the pathogenesis of diabetes. Homeostasis of Zinc is thought to depend on absorption as well as excretion. Studies have shown that the Zinc ingested by healthy persons are eliminated in the feces (90%) and in urine (2–10%) [8]. Zinc is primarily absorbed from small intestine, duodenum and ileum [9]. The oral Zinc tolerance test was proved to be an acceptable method to study zinc absorption and excretion in humans [10]. Absorption and/or excretion of Zinc may be altered in various pathological states, such as diabetes mellitus. Pre-diabetes is an intermediate state of hyperglycemia with glycaemic parameters above normal but below the threshold for the initiation of treatment for diabetes [11]. The pre-diabetic state is characterized by either impaired

glucose tolerance (IGT), impaired fasting glucose (IFG) or both. In a meta-analysis evaluating the progression of pre-diabetes to diabetes published in 2007, the annual incidence rate of diabetes was found to be 4%-6% for isolated IGT, for isolated IFG 6%-9% and for both IGT and IFG was 15%-19% [12]. Presently there are no studies examining the pharmacokinetic properties of Zinc in pre-diabetes. Understanding Zinc metabolism in the pre-diabetic state could help to define the role of Zinc in the pathogenesis of diabetes mellitus. Hence the present study aims to investigate the pharmacokinetic parameters of Zinc in pre-diabetes using the oral Zinc tolerance test.

Methods

The present study was conducted as a pharmacokinetic sub-study of a randomized controlled trial evaluating the effects of Zinc supplementation in pre-diabetes [13]. Ethical approval for the study was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo.

Study population

The study comprised of 10 individuals with pre-diabetes who were in the Zinc treatment arm of the above clinical trial. The inclusion and exclusion criteria for the clinical trial are described elsewhere [13]. In summary, to be included in the study the participants had to be:

I. between the ages of 18–60 years, eligible for study through a screening test confirming the presence of pre-diabetes,

II. not on any other vitamin or mineral supplementations or the current use of a weight loss medicine or dietary modification,

III. having normal hepatic or renal functions,

IV. non-lactating and non-pregnant. Subjects who were in the Zinc (20mg) treatment arm for at least 1 week in the above trial were invited for the pharmacokinetic sub-study. Informed written consent was obtained from each participant prior to recruitment for the study, after the nature, purpose, inconveniences, risks and benefits of participation were explained. Subjects were compensated for their participation. This study was conducted at the Department of Pharmacology, Faculty of Medicine, University of Colombo, Sri Lanka in compliance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines.

Data collection

The participants were asked to come after 10 hours of overnight fasting. On the day of the study, initially a baseline blood sample was taken 8.00am (0hrs). This blood sample was obtained 24 hours after their regular Zinc dose, taken in the morning of the preceding day as a part of the clinical trial. The Zinc 20mg capsule (Zinc Sulphate.H_20, Astron Lanka Ltd, Colombo, Sri Lanka) was given to the patient to be taken orally after obtaining this baseline blood sample (0hrs). Subsequent blood samples were taken at 30 min, 1h, 2h, 3h, 6h and 8h. A 24 hour sample was taken in the morning of the following day at 8.00am. Each blood sample was about 2-2.5ml, and was obtained from the ante-cubital fossa under sterile conditions. Subjects were given standard meals during the period of the study. This included a breakfast (rice) given at 3 hours, and two subsequent snacks (biscuits) given at 6 hours and 9 hours, followed by a standard dinner (string hoppers) was given at 12 hours.

Food items with a high Zinc content were avoided in preparation of these meals. The subjects rested in bed throughout the test. In addition to the above the subjects basic anthropometric details, including height, weight and body mass index (BMI) were measured. Body weight was measured using a calibrated electronic floor scale (SECA 815 by SECA GmbH & Co. Kg. Hamburg, Germany) to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using an upright plastic portable Stadiometer (SECA 217 by SECA GmbH & Co. Kg. Hamburg, Germany). BMI was calculated as weight (in kilograms) divided by the square of height (in meters). All anthropometric measurements were made by using standard equipment and following WHO guidelines. Seated blood pressure (Systolic and Diastolic) was measured after a 10-min rest with Omron IA2 digital blood pressure monitors (Omron Healthcare, Singapore)

Biochemical analysis of serum zinc

The centrifuged serum separated samples (2200-2500 rpm) were tested at the Department of Pharmacology, Faculty of Medicine, University of Colombo, Sri Lanka. Serum Zinc was analyzed by colorimetric method in Mindray BA-88A semi auto-analyzer (Mindray medical International LTD, China) using commercially available colorimetric determination kits of serum Zinc. This is a direct colorimetric assay based on the 5-Br-PAPS method. The Zinc determination is based on the reaction of Zinc with 5-Br-PAPS at alkaline pH in a buffered media, which forms a stable colored complex. The color intensity is proportional to the Zinc concentration in the sample. Absorbance of the Zn^{2+}-complex is measured at 560nm. The results are reported as micromoles per liter (µmol/l).

Quantitative analysis of the zinc capsules

Twenty capsules of Zinc containing Zinc sulphate monohydrate ($ZnSO_4.H_20$) were chosen at random for analysis. Zinc content was determined by Atomic Absorption Spectrophotometry method AOAC 999.11 (2012) (AOAC International, Maryland, USA) [14]. The tests were carried out at the Residue Analysis Laboratory, Industrial Technology Institute (ITI), Colombo, Sri Lanka. The results are reported as mg of Zinc per capsule.

Pharmacokinetic evaluation

Pharmacokinetic parameters were calculated using the non-compartment extra-vascular model applied for the Zinc plasma disposition curves. The area under the plasma concentration-time curve (AUC) was calculated by use of the mixed log-linear trapezoidal method. Values for the maximum plasma concentration (Cmax) and time to peak plasma concentration (Tmax) were directly determined from the plasma concentration –time curve. The elimination half-life (T½) was calculated as 0.693/Ke, in which Ke was the elimination rate constant calculated based on sample taken at 2hrs, 3hrs, 6hrs and 8hrs.

Statistical analysis

Parametric and non parametric statistical tests were done using the SPSS version 14 (SPSS Inc., Chicago, IL, USA). Dichotomous variables are reported as numbers and percentages and compared using chi-square test. Continuous variables are presented as means ± standard deviation and intergroup comparisons were conducted with Student's t-test or ANOVA with post-hoc analysis. In all analyses a $p < 0.05$ was considered as statistically significant.

Results

Sample size was 10, of which four patients were males. Mean age (±SD) was 52.6±9.6 years. Characteristics of the study population, including age, weight, height, body mass index, systolic and diastolic blood pressure, and initial serum Zinc concentration prior to the commencement of Zinc supplementation in the clinical trial are provided in (Table 1). The serum Zinc concentration of all study participants with pre-diabetes prior to the commencement of Zinc supplementation in the clinical trial were below the normal range for serum Zinc (15.29 – 21.41 µmol/l).

Pharmacokinetics of zinc

The mean serum Zinc concentration (±SD) at baseline (0 hours) was 10.63±3.0 µmol/l, which was higher than the mean pre-supplementation Zinc concentration (9.06±1.93 µmol/l) (p=0.10). The change in mean serum Zinc concentration (±SD) with time, using time zero as the reference point is shown in (Figure 1). Zinc concentration increased from baseline (0 hours) and the maximal concentration of Zinc (±SD) achieved in the blood (Cmax) was 23.56±4.46 µmol/l. The time taken to reach the maximal Zinc concentration (Tmax) was 2 hours. Subsequently the Zinc concentration gradually decreased, however at 24 hours an increase in the mean Zinc concentration was observed (Figure 1). This was also observed independently in all study participants (data not shown). The area under the curve (AUC) from time of administration to the time of last observation was 145.31 µmol h^{-1} l^{-1}. AUC_{∞} (area under the concentration with time curve extrapolated to infinity) was 228.21 µmol h^{-1} l^{-1}. The elimination rate constant (Ke) was 0.14 h^{-1}. The elimination half life (T½) was 4.91 hours.

Table 1: Characteristics of the Study Population.

Subject	Age (Years)	Weight (kg)	Height (m)	BMI (kg/m²)	SBP (mmHg)	DBP (mmHg)	Serum Zinc* (µmol/l)
1	64	61.5	1.59	24.33	130	90	11.17
2	59	76.1	1.61	29.36	120	90	11.44
3	49	43.7	1.55	18.19	110	80	10.26
4	57	55.8	1.49	25.13	150	90	9.92
5	55	57.3	1.47	26.52	130	90	7.18
6	62	49.9	1.52	21.6	110	70	5.51
7	34	68.6	1.73	22.92	120	90	10.01
8	42	71.6	1.72	24.2	126	82	9.21
9	46	83.2	1.64	30.93	131	77	7.01
10	58	60.3	1.62	22.98	114	81	8.94
Mean	52.6	62.8	1.59	24.62	124.1	84	9.06
SD	9.6	12.1	0.7	3.7	12.1	7.1	1.9

BMI: Body Mass Index; DBP: Diastolic Blood Pressure; SBP: Systolic Blood Pressure; SD: Standard Deviation
*Serum Zinc prior to initiation of Zinc supplementation

Zinc content of the capsules

The specified Zn content per capsule on the package label was 20 mg. The average content measured by Atomic Absorption Spectrophotometry method AOAC 999.11 (2012) (AOAC International, Maryland, USA) was 21 mg per capsule.

Discussion

This is the first study evaluating the pharmacokinetics of Zinc in those with pre-diabetes. Our results show that all ten participants with pre-diabetes had their baseline serum Zinc levels below the normal level at the initiation of the clinical trial. The presence of hypozincaemia in Type-2 diabetes has been demonstrated by numerous studies conducted during the past two decades [6,15]. Several studies have also demonstrated a relationship between serum Zinc levels and glycaemic control in patients with Type-2 diabetes, and an improvement in glycaemic control with short-term Zinc supplementation [15,16]. However, whether hypozincaemia precedes diabetes or results from altered Zinc metabolism due to diabetes has been much debated. The most recent evidence, including the present study suggests that hypozincaemia is also present even in those with pre-diabetes, hence preceding the onset of Type-2 diabetes [17]. Furthermore, Zinc supplementation has also been shown to improve glucose handling in pre-diabetes [18]. Zinc deficiency is known to have numerous detrimental health effects [19]. The concentration of Zinc in plasma or serum is currently the best available biomarker of Zinc deficiency in a population [19]. Numerous Zinc

supplementation trials have shown that a wide range of health benefits can be realized by increasing the intake of Zinc where diets are inadequate in this micronutrient [20]. Initiation of Zinc supplementation in the present study group as a part of the clinical trial, resulted in an improvement in their Zinc levels, as evidenced by the rise in the serum Zinc concentration. Although this increase was statistically non-significant, possible owing to the smaller sample size, Zinc supplementation could be used as a low cost intervention to improve Zinc status in populations who are Zinc deficient. Previous studies have noted a Tmax for Zinc sulphate between 2-3 hours, a finding consistent with that observed in the present study [21]. Furthermore, we observed an elimination half life of 4.91 hours, and previous studies have demonstrated an elimination half life of 0.78-2.63 hours among healthy adults [21]. Hence, absorption of Zinc in those with pre-diabetes, seems to be similar to that observed among healthy adults, however elimination half life seems to be prolonged. We also noted a rebound increase in the serum Zinc concentration at 24 hours. This is possibly due to the entero-hepatic re-circulation which is known to be present for Zinc [21]. The re-circulation of pharmacological doses of Zinc is consistent with a physiological mechanism for enter-hepatic re-circulation of Zinc [22].

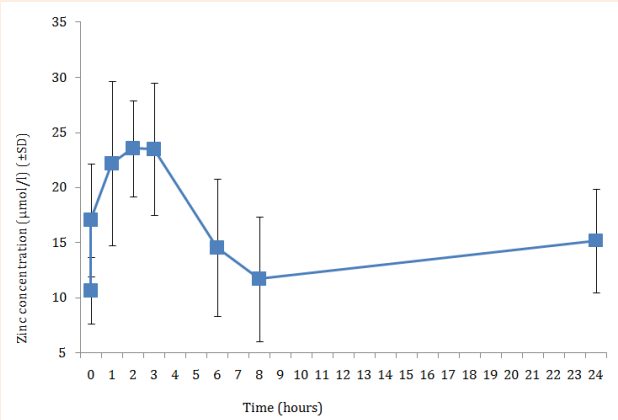

Figure 1: Zinc concentration vs. time curve.

Substantial evidence has indeed accumulated showing that endogenous Zinc is returned to the digestive tract mainly via pancreatic and biliary secretions or by direct transfer from cells of the intestinal wall [22]. These secretions are considered as an important mechanism for Zinc homeostasis [22]. However, it is important to note that previous studies have observed more frequent cycles of enteral re-circulation for Zinc, with the first rebound effect appearing after 1.4 hours during the absorption phase, with subsequent cycles occurring at regular intervals of 1.2 hours [22]. However, this was not observed in the present study, and hence the presence of hypozincaemia in the present pre-diabetic population could be a result of the impaired and slow physiological enteral re-circulation, which could also be the reason for the prolonged elimination half life. However, future studies need to carefully look at the entero-hepatic circulation in pre-diabetes by increasing the number of samples during the elimination phase.

Studies have shown that Zinc deficiency is considerably prevalent in developing nations of the South Asian region [23]. Another important reason for the observed Zinc deficiency in this population with pre-diabetes could be inadequate dietary intake. Several studies confirmed that inadequate intake of Zinc is one of the most significant determinants for the development of Zinc deficiency [24]. Furthermore, it is important to note that almost all studies showing a beneficial effect of Zinc supplementation on glycaemic control, are from Asian countries [25]. Hence, Zinc deficiency could be one of the reasons for the observed high prevalence of Type 2 diabetes in the region [26]. Regarding the limitations of this study, because urinary Zinc concentrations were not measured, we cannot comment on the renal clearance of Zinc in the present study population. Furthermore, the lack of a control group is also a limitation.

Conclusion

Our results show the presence of hypozincaemia in those with pre-diabetes, which was improved with Zinc supplementation. Zinc absorption was normal in the study population, however elimination half life was prolonged. Furthermore, there is possible impairment in entero-hepatic re-circulation observed in this population with pre-diabetes.

Declarations

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgement

Not applicable.

Authors' contributions

PR, RJ, PG, GRC and PK made substantial contribution to conception and study design. PR, CD and AL were involved in data collection. PR, RJ, CD and AL were involved in refining the study design, statistical analysis and drafting the manuscript. PR, PG, PK and GRC critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Review Committee, Faculty of Medicine, University of Colombo, Sri Lanka. Informed written consent was taken from each participant.

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Availability of data and material

Please contact author for data requests.

Consent for publication

Not applicable.

References

1. King JC, Cousins RJ (2006) Zinc. In: Shils ME & Shike M (Eds.), Modern Nutrition in Health and Disease (10th edn), Lippincott Williams and Wilkins, USA, pp: 271-285.

2. Chausmer AB (1998) Zinc, insulin and diabetes. J Am Coll Nutr 17(2): 109-115.

3. Brandao-Neto J, da Silva CA, Figueiredo NB, Shuhama T, da Cunha NF, et al. (1999) Lack of acute zinc effects in glucose metabolism in healthy and insulin-dependent diabetes mellitus patients. Biometals 12(2): 161-165.

4. Brandão-Neto J, Vieira JGH, Shuhama T, Russo EMK, Piesco RV, et al. (1990) Interrelationships of zinc with glucose and insulin metabolism in humans. Biological Trace Element Research 24(1): 73-82.

5. Pidduck HG, Wren PJ, Evans DA (1970) Hyperzincuria of diabetes mellitus and possible genetical implications of this observation. Diabetes 19(4): 240-247.

6. Garg VK, Gupta R, Goyal RK (1994) Hypozincemia in diabetes mellitus. J Assoc Physicians India 42(9): 720-721.

7. Kinlaw WB, Levine AS, Morley JE, Silvis SE, McClain CJ (1983) Abnormal zinc metabolism in type II diabetes mellitus. Am J Med 75(2): 273-277.

8. Hambidge KM, Casey CE, Krebs NF (1986) Zinc. Academic Press, USA.

9. Sorensen JA, Andersen O, Nielsen JB (1998) An in vivo study of the gastrointestinal absorption site for zinc chloride in mice. J Trace Elem Med Biol 12(1): 16-22.

10. Brandão-Neto J, Silva CAB, Rezende AA, Almeida MG, Sales VSP, et al. (2003) Zinc pharmacokinetics in insulin-dependent diabetes mellitus patients after oral zinc tolerance test. Nutrition Research, 23(2): 141-150.

11. Bansal N (2015) Prediabetes diagnosis and treatment: A review. World J Diabetes 6(2): 296-303.

12. Gerstein HC, Santaguida P, Raina P, Morrison KM, Balion C, et al. (2007) Annual incidence and relative risk of diabetes in people with various categories of dysglycemia: a systematic overview and meta-analysis of prospective studies. Diabetes Res Clin Pract 78(3): 305-312.

13. Ranasinghe P, Jayawardena R, Pigera A, Katulanda P, Constantine GR, et al. (2013) Zinc supplementation in pre-diabetes: study protocol for a randomized controlled trial. Trials 14(1): 52.

14. Official methods of analysis (1999).

15. Saharia GK, Goswami RK (2013) Evaluation of serum zinc status and glycated hemoglobin of type 2 diabetes mellitus patients in a tertiary care hospital of assam. J Lab Physicians 5(1): 30-33.

16. Oh HM, Yoon JS (2008) Glycemic control of type 2 diabetic patients after short-term zinc supplementation. Nutr Res Pract 2(4): 283-288.

17. Islam MR, Arslan I, Attia J, McEvoy M, McElduff P, et al. (2013) Is Serum Zinc Level Associated with Prediabetes and Diabetes?: A Cross-Sectional Study from Bangladesh. PLoS ONE 8(4): e61776.

18. Islam MR, Attia J, Ali L, McEvoy M, Selim S, et al. (2016) Zinc supplementation for improving glucose handling in pre-diabetes: A double blind randomized placebo controlled pilot study. Diabetes Res Clin Pract 115: 39-46.

19. Roohani N, Hurrell R, Kelishadi R, Schulin R (2013) Zinc and its importance for human health: An integrative review. J Res Med Sci 18(2): 144-157.

20. Maret W, Sandstead HH (2006) Zinc requirements and the risks and benefits of zinc supplementation. J Trace Elem Med Biol 20(1): 3-18.

21. Neve J, Hanocq M, Peretz A, Abi Khalil F, Pelen F, et al. (1991) Pharmacokinetic study of orally administered zinc in humans: evidence for an enteral recirculation. Eur J Drug Metab Pharmacokinet 16(4): 315-323.

22. Cousins RJ (1985) Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiological reviews 65(2): 238-309.

23. Akhtar S (2013) Zinc status in South Asian populations--an update. J Health Popul Nutr 31(2): 139-149.

24. de Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C (2007) Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. Food and nutrition bulletin 28(3 Suppl): S480-484.

25. Jayawardena R, Ranasinghe P, Galappatthy P, Malkanthi R, Constantine G, et al. (2012) Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. Diabetol Metab Syndr 4(1): 13.

26. Jayawardena R, Ranasinghe P, Byrne NM, Soares MJ, Katulanda P, et al. (2012) Prevalence and trends of the diabetes epidemic in South Asia: a systematic review and meta-analysis. BMC Public Health 12: 380.

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