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. 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 10.65±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
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. 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 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 $\text{Zn}^{2+}$-complex is measured at 560nm. The results are reported as micromoles per liter ($\mu$mol/l).

Quantitative analysis of the zinc capsules

Twenty capsules of Zinc containing Zinc sulphate monohydrate (ZnSO$_4$·H$_2$O) 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).

Table 1: Characteristics of the Study Population.

<table>
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<tr>
<th>Subject</th>
<th>Age (Years)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>BMI (kg/m²)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Serum Zinc* (μmol/l)</th>
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</table>

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