Original article

Effect of Furosemide and Spironolactone on urinary zinc excretion in rats

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ABSTRACT

Background: Zinc deficiency is associated with numerous diseases including hypertension, diabetes, obesity, immune dysregulation, cancer, depression and congenital anomalies. There are many reasons of zinc deficiency including some medications. If zinc supplementation is used with these medicines than many diseases can be prevented.

Subjects and methods: This experimental study was planned to observe the effect of single diuretic dose of furosemide and spironolactone on zinc urinary excretion and blood levels in normal rats. Eighteen adult healthy male Sprague Dawley rats were randomly divided into three groups. After saline load rats were given distilled water, furosemide (10 mg/kg) and spironolactone (20 mg/kg) as single oral dose. Blood and urine samples were collected after five hours and analysed for zinc concentration by flame atomic absorption spectrophotometer.

Results: Single oral dose of furosemide and spironolactone highly significantly increased urinary zinc excretion (p-value <0.001 vs normal control), and increased blood zinc level (p-value <0.001 *vs.* normal control). Value of both variables were significantly higher in furosemide-treated group (p-value <0.001 *vs.* furosemide-treated).

Conclusion: Results of this research conclude that furosemide and spironolactone increase urinary zinc excretion when used for short period. It is also postulated that blood zinc concentration is not reliable measure to assess the zinc status of the body because its level shows compensatory rise during deficiency states.

Keywords:

Furosemide, Spironolactone, Urinary zinc excretion, Blood zinc concentration, Rats

INTRODUCTION

Zinc is sixth most abundant metal in human body and most widely used in enzymes.[1] Zinc is critical component of almost 10% of genomic proteins and plays important role in functioning of over 300 enzymes. It also has an antioxidant role, being a critical component of superoxide dismutase, peroxidase, catalase[2] and glutathione peroxidise.[3] It protects the cell membranes, microtubules, tubules, enzymes, DNA and proteins by scavenging free radicals and protecting from oxidative damage.[4] Zinc being essential part of 'zinc finger protein' (finger like structure of the transcriptional regulatory protein), that interact with DNA and RNA and regulates transcription and replication, regulates growth and development of cell.[5] Local concentration of zinc determines the life span of a cell by either acting as inducer or inhibitor or protector of apoptosis.[4,6] Zinc concentration of plasma is only <0.1%.[7] Around 90% of blood zinc is present in red blood cells in the form of metal component of superoxide dismutase and carbonic anhydrase enzyme. Despite small percentage of plasma zinc, it is utterly important because it reflects whole body zinc and no sign and symptoms of zinc deficiency appear until and unless plasma zinc concentration drops.[8] Gastrointestinal excretion is the main route of zinc excretion[9] followed by urinary route which is responsible for only 10% of it and others are minor routes.[9,10] Deficiency of zinc in body can be due to decreased intake and absorption of zinc, increased loss of zinc due to chronic renal failure, Acetyl Cholinesterase (ACE) inhibitors use and hepatic disease. Increased requirement occurs during proliferative skin diseases, cancer, chronic infection, stress, growth, pregnancy and lactation.[11-18] Deficiency of zinc is associated with multiple diseases like immune dysregulation, hypertension, diabetes, congenital anomalies, sickle cell anemia and rheumatoid arthritis.[19-24] Furosemide and spironolactone have proven role in urinary excretion of Na, Cl and Mg but there is no definitive opinion on its effect on urinary zinc excretion.[25,26] This study aims to elaborate furosemide and spironolactone effect on urinary zinc excretion.

Conflict of interest: The authors declared no conflict of interest exists.

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SUBJECTS AND METHODS

Study was conducted at Post Graduate Medical Institute Lahore and Government College University Lahore from Marchto May 2018 after approval from institutional ethical committee. Eighteen adult male Sprague Dawley rats of 105-127 g weight and 6-7 weeks of age were included in study and rats showing signs of any disease were excluded. Animals were bred in the animal house of post graduate medical institute and kept in same animal house. Study was started after one week of acclimatization. Initial selection was according to above mentioned inclusion and exclusion criteria and divided into normal control, furosemide-treated and spironolactone-treated groups by simple random sampling using lottery method. On the day of experiment each rat was fasted 18 hours and given 25 ml/kg normal saline orally before drug administration.[27] Rats in normal control group were given distilled water in an amount equal to the amount administered to the experimental group, furosemide-treated rats were given furosemide 10mg/kg and spironolactone-treated rats were given spironolactone20mg/kg orally as a single morning dose and kept in metabolic cage without access to food and water.[28,29] Urine was collected for 5 hours, measured, centrifuged and supernatant was stored at -20ºC. After urine collection, one ml blood sample was collected by cardiac puncture under light anaesthesia and put in EDTA vacutainer and stored at -20ºC. Zinc analysis of samples was done by flame atomic absorption spectrometer (AA-TOOOF Shimadzu, made in Japan) by using wet digestion method[29] in Chemistry Department of Government College University, Lahore. The data collected was analyzed using Statistical Package for Social Sciences (SPSS 22) and graphic representation by graph pad prism 5. After checking normal distribution of data by Shipiro Wilk test, it was presented as mean ± standard deviation (SD). ANOVA was applied to test significance among groups and post hoc Tukey's test was applied to observe which group mean differs. A p-value of ≤0.001 was considered statistically very highly significant, ≤0.01 was highly significant and ≤0.05 was significant.

RESULTS

Body weight (Mean ± SD) of the rats was 105±3 g, 127±4 g and 118±3 g in normal control, furosemide-treated and spironolactone-treated groups respectively. Furosemide-treated group had the highest values of urinary zinc concentration, urine volume and total zinc excretion followed by spironolactone-treated group, while normal control group had the lowest values. A p-value of <0.001by ANOVA showed that the differences among the groups were highly significant in all of three parameters. Post hoc multiple comparison showed that furosemide and spironolactone-treated group had significantly higher urinary zinc concentration, urine volume and total zinc excretion as compared to normal control group, while furosemide-treated group had significantly higher levels as compared to spironolactone-treated group (Figure 1 A, B, C).

Furosemide-treated group had the highest level of blood zinc concentration followed by spironolactone-treated group, while normal control group had the lowest blood zinc concentration. A p-value of <0.001 by ANOVA showed that the difference among groups was very highly significant (Figure 1 D). Post hoc multiple comparison showed that furosemide and spironolactone-treated groups had significantly higher blood zinc level as compared to normal cont.

DISCUSSION

Zinc is an important constituent of metalloenzyme in humans, required for multiple physiological functions, growth and normal development[31] and its deficiency is associated with multiple diseases like hypertension, diabetes, myocardial infarction, immune dysregulation, depression and neoplasia.[19-21,31-34] Furosemide is used for the treatment of edematous conditions, heart failure, hypertension, chronic liver disease and acute renal failure and spironolactone is used for the treatment of congestive cardiac failure, cirrhosis of liver and in combination for the treatment hypertension.[25,26] There emerges a question that if furosemide and spironolactone result in urinary zinc excretion, then their efficiency for treating these diseases may be affected and also can be cause of causing other ailments until or unless zinc supplementation is given. But the effect of these diuretics on zinc homeostasis is not yet established. To make it clear this study was planned to observe the effect of single dose of furosemide and spironolactone on urinary zinc excretion in healthy rats. For this purpose, lipschitz test was used, and blood and urine zinc levels were estimated by flame atomic absorption spectrophotometer.[27] Blood zinc concentration reflects serum zinc concentration because zinc pool in red blood cells exchanges readily with plasma zinc in less than one hour. The study results show that furosemide-treated group has significantly highest levels of urinary zinc concentration, urine volume and total zinc excretion, followed by spironolactone-treated group, while lowest in normal control group. Cysteine infusion study conducted on anesthetized dogs showed that zinc appears in urine by

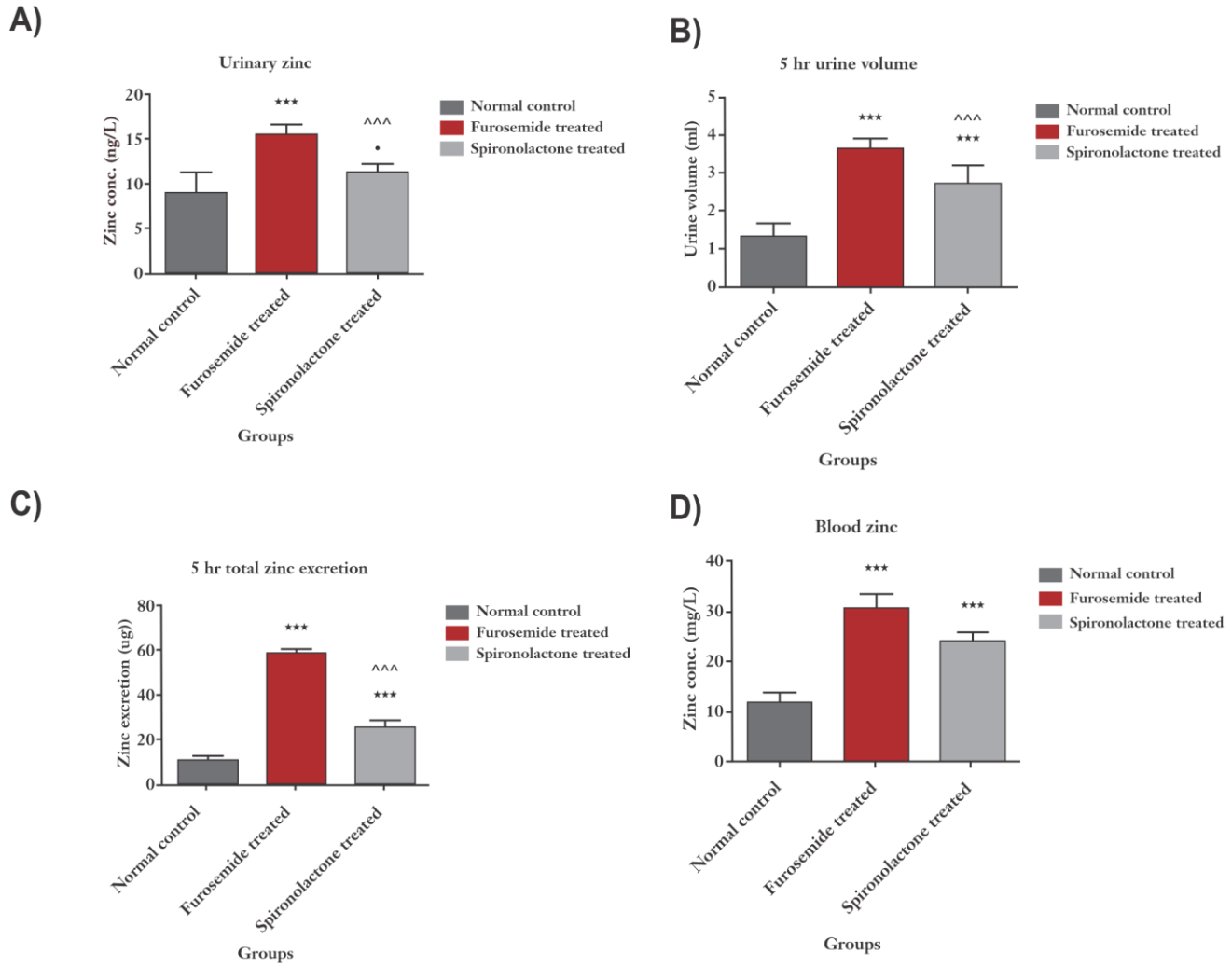

Figure 1. Effect of furosemide and spironolactone on (A) on urinary zinc excretion, (B) Five hours urine volume, (C) five hours total zinc excretion and (D) blood zinc level in rats. Data presents mean ± SD (n=6); *** p-value<0.001, * p value<*0.05* (vs. Normal control).*** p-value<0.001 (vs. Furosemide-treated).

glomerular filtration and proximal tubular secretion and reabsorbed through distal tubule.[35] High urinary zinc concentration in present study can either be due to increased glomerular zinc filtration or high zinc secretion in proximal tubule or decreased reabsorption through the distal tubules or a combination of these effects. In zinc sulphate infusion study it was found that the zinc gets reabsorbed through distal tubules just like the reabsorption of NaCl.[35] A human study conducted on liver cirrhosis patients, creatinine adjusted urinary zinc excretion was calculated and concluded that furosemide and spironolactone increase urinary zinc excretion by reducing reabsorption of zinc by distal tubules.[36] Another animal study observed that furosemide increased urinary zinc excretion in dose dependent manner.[37] In this study excess urinary zinc excretion is a combined result of high urine volume and high zinc concentration of urine. High volume is due to the diuretic property of the drugs and high urinary zinc concentration is probably due to decreased reabsorption of zinc from distal tubule. In present study, there is also significant difference in urinary zinc excretion between furosemide and spironolactone-treated group. One possible cause of this difference is that both furosemide and spironolactone have different mechanism and site of action in renal tubules and reabsorption of zinc is correlated to reabsorption of NaCl.[35,36] So both agents exert different effect on renal tubular reabsorption of zinc. Furosemide causing more natriuresis, causes more zinc loss.

In our study blood zinc concentration is significantly higher in furosemide-treated group

followed by spironolactone-treated group, while lowest in normal control group.[39] A study long term conducted on patients of liver cirrhosis, both serum zinc and urinary zinc levels were higher in group that received diuretics (spironolactone and furosemide) with zinc preparation when compared with groups which received only zinc or diuretics. That rise in blood zinc level was due to increase zinc absorption from gastrointestinal tract in an attempt to maintain serum zinc level.[36] Zinc deprivation studies prove that during short term zinc deprivation serum zinc is maintained by removal of zinc from rapidly exchangeable zinc store but during long term zinc deprivation increased gastrointestinal zinc absorption comes into play to maintain serum zinc level.[38] Higher blood zinc concentration despite increased urinary zinc excretion after single diuretic dose in present study may be due to redistribution of zinc from body stores to compensate excessive urinary loss. Rapidly exchangeable store is liver but hepatic zinc is not estimated in this study. Other limitations are very short duration of study using single diuretic dose in only one animal species only. Liver store which is rapidly exchangeable store of zinc that is not measured.

CONCLUSION

From results of this research, it may be concluded that furosemide and spironolactone increase urinary zinc excretion when used for short period and blood zinc concentration is not reliable measure to assess the zinc status of the body because its level shows compensatory rise during deficiency states Zinc supplements are recommended along with these diuretics to compensate the urinary zinc loss. Animal and human long duration studies using these diuretics measuring zinc absorption and body tissue content are recommended to further elaborate the effect of these diuretics on zinc homeostasis.

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