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Original Article

Ameliorative Effect of Zinc on Bone Cells of Humerus and Femur of Female Rats Under High Salt Diet

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Abstract

Background:To evaluate the effect of zinc on bone cells of humerus and femur of female adult Sprague Dawley rats under high salt diet.

Methods: In this experimental study 10-12 weeks old,45 adult female Sprague Dawley rats were used. Three groups were made, each having fifteen rats. Control group C received laboratory diet without any alteration. Experimental group A was served with high salt diet (8%NaCl)whereas experimental group B animals were given high salt diet augmented with zinc (50mg/kg/day).All groups were given the diet for eight weeks after which they were sacrificed and left humeri and femora of all rats were obtained.After decalcification, Bone tissue from proximal part of shaft was attained to study the number of osteoblast (bone forming) and osteoclast (bone resorbing) cells.For light microscopy, tissue processing was done to obtain five micrometer (µm) sections. Tissues were stained with Haematoxylin and eosin (H&E).The results were compiled and compared.

Results: After salt administration, group Animals showed marked increase in osteoclast number whereas the osteoblast number exhibited substantial decrease. Protective effects were seen in zinc supplemented experimental group B with decrease in osteoclast number and increase in osteoblast number.

Conclusion:Zinc has protective effect against high salt induced damage in the bone cells of rats.

Key words: Osteoblast, Osteoclast, Salt, Zinc.

Introduction

Osteoporosis is a progressive, metabolic and degenerative disease of the bones characterized by micro architectural defects, bone mass reduction and decrease resistance to mechanical injuries. Over 2000 million people, one in every tenth person in the world is its victim.[1-5] It reflects a disparity between bone formation and bone resorption hence increasing skeletal turnover and bone fragility.[6-7] Osteoblasts, mesenchymal in origin, flourish and discriminate prior to bone formation and are present on the forming surfaces of growing or remodeling bone. Multinucleated osteoclasts are derived from circulating precursors of the monocyte-macrophage cell line and are bone resorbing cells which require receptor activator of nuclear factor kappa-B ligand(RANKL) and Macrophage colony stimulating factor . [8-9] Healthy bone depends on the balanced activities of bone cells. Shift of balance results in cluster of abnormalities in which bones have low mass and altered microstructure leading to increase fracture risk.[10,11]

Salt is one of the oldest and most ubiquitous of food flavorings and within recommended levels, is necessary for lives of organisms. International recommendations suggest that average population intake should be less than 5-6 g whereas most adult populations have exceeded the nutritional recommendations of salt intake average being 6-12g.[12, 13] Increase salt in diet can disrupt the equilibrium of formation and resorption of bone ultimately resulting in increased excretion of sodium in urine along with calcium which in turn stimulates bone resorption activities.[14, 15] Increase sodium intake is a risk factor for osteoporosis.[(16, 17)]due to change in bone mineral density and detrimental effect on calcium homeostasis.[16-19] Zinc is a component of more than 200 enzymes and 23rd most abundant element in the earth's crust with enzymatic function. [11,20] It reduces osteoclast resorption activities and increase markers of osteoblast differentiation, matrix maturation and mineralization.[21] Zinc may increase bone formation through stimulating cell proliferation, alkaline phosphatase activity and collagen synthesis.[22] Zinc inhibits bone loss by bone protein synthesis,and exerts beneficial effect on IGF-I and TGF-β1 production in the bone tissues[23, 24]

Osteoclasts are exquisitely sensitive to zinc which is a highly effective inhibitor of bone resorption.[25] It has been shown that zinc can increase the production of osteocalcin and stimulate the proliferation and function of osteoblastic cells in bone tissues. [26,27] Therefore, zinc can influence the skeletal growth by

stimulating osteogenesis, escorted by a parallel inhibition of osteoclastogenesis.[28] It can also induce matrix formation, mineralization in bone and increase in osteoid area by augmenting collagen production.[22,29-31] It has been observed that zinc can inhibit the differentiation of osteoclasts and remote osteoblast activity,thus affecting the formation of hard tissues. [25,31,32]

Material and Methods

This experimental study was conducted in Anatomy department of Islamic International Medical College and National Institute of Health (NIH) Islamabad. Forty five Weighing 250-300 grams, 12weeks old female Sprague Dawley rats were used for research. Three groups of animals were assembled, fifteen rats per group.Temperature of 20-26°C and well ventilated room was provided for animals to get used to new environment. Group C served as controls and they received standard laboratory diet with tap water as their drink.Group A were fed on diet having 8% NaCl for eight weeks whereas group B were fed on diet containing salt supplemented with zinc at a dose of 50mg/kg body weight.[33,34] Water was provided ad libitum. The dose of NaCl and Zinc was set according to previous studies. Animals were dissected after eight weeks. The left femora and humeri were removed and 10% neutral buffered formaldehyde was utilized for fixation for2 days.Aqueous solution of 5-10% nitric acid was used for decalcification for 24-48 hours.Longitudinal sections from proximal femur just below the greater trochanter were obtained for the study of bone cells, processedand embedded in paraffin wax to form blocks.Blocks were mounted on rotary microtome to obtain sections having thickness of 5µm.Haematoxylin and eosin was usedfor ordinary histological study.The number of osteoblasts in humeri and femora was counted at magnification of X4. They were counted with the help of square of eyepiece, per unit area, in four different random non overlapping fields of trabecular bone . [8] Osteoclasts number in humeri and femora were counted in ten random fields of each slide at magnification of X4.The counting was done in trabecular bone of the proximal end of femur just below the greater trochanter and the readings were then averaged.[35-37] Intra-group comparison was done with t-test. One Way Analysis of Variance (ANOVA) and Post hoc tukey test was applied for inter group comparison.Qualitative data was assessed by applying Pearson Chi Square test.*p*-value <0.05 was considered statistically significant.

Results

The mean value of osteoblasts in Humerus for group C was 7.300±0.78, number of osteoblast was reduced to 473±0.452 in group A while an increment was seen in group B, 6.900±0.840(p<0.05) (Table 1). Multiple comparison revealed a significant difference of 1.826 between group C and A(*p*=0.000). Between group C and B, value of 0.400 was obtained (*p*=0.286) and was statistically unimportant. The difference of osteoblasts number between group A and B was -1.4266 (*p*=0.000) (Table 2).For osteoblasts number in Femur of group C, value of 8.586±0.966, was recorded. The osteoblast count was 6.806±0.928 in group A and 8.033±0.703 in group B. The results were significant(*p*<0.05).The difference between group C and group A was 1.780 (*p*=0.000) whereas statistically negligible result between group C and B was observed (*p*=0.205). The difference of number between group A and B was -1.226 (*p*=0.001) indicating beneficial effects of zinc in group B(Table 2).Results clearly indicated the decrease in number of osteoblasts in group A(Fig 2)as compared to group C (Fig 1) and increase in number of osteoblast due to zinc in group B (Fig 3).

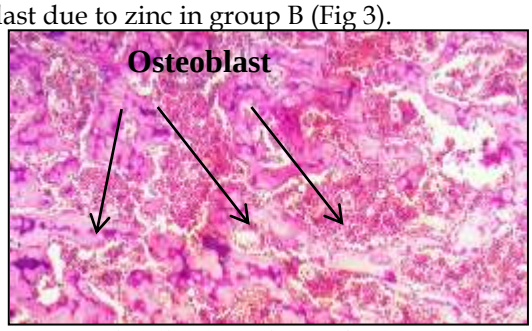

Figure 1: Longitudinal-section of Femur showing abundanat osteoclasts

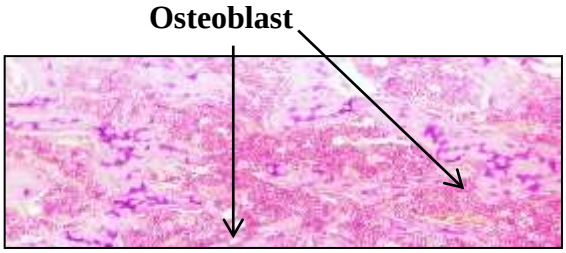

Figure 2: Longitudinal-section of Femur of A9 showing Osteoblasts (OB). H&E, X100.

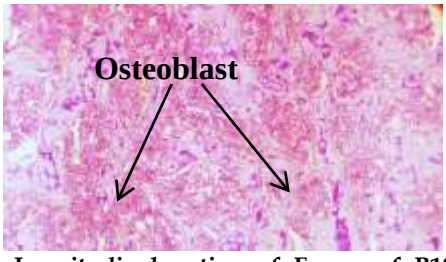

Figure 3: Longitudinal-section of Femur of B10 showing Osteoblasts (OB). H&E, X100.

Table 1: Mean Osteoblast number per unit area in humerus and femur of all groups

	Humerus			Femur		
Groups	C	A	B	C	A	B
Mean Score	7.300	5.473	6.900	8.586	6.806	8.033
Std. Deviation	0.7883	0.4527	0.8409	0.9664	0.9284	0.703
SEM	0.2035	0.1168	0.2171	0.2495	0.2397	0.18
p-vallue	0.000*			0.000*		

Table 2: Multiple comparison of mean Osteoblast number per unit area in humerus and femur among all groups

	Humerus			Femur		
Groups	C vs. A	C vs. B	A vs. B	C vs. A	C vs. B	A vs. B
Mean Difference	1.826	0.4000	-1.4266	1.7800	0.5533	-1.2266
p value	0.000*	0.286	0.000*	0.000*	0.205	0.001*

Mean value of osteoclasts in Humerus for group C was 2.666±1.046, 4.400±0.985 for group A and 2.600±1.242 for group B (Table 3). All groups showed variable count of osteoclasts (p=0.000).Intercomparison between Group C and A revealed significant value (p=0.000). Furthermore, group A had more number of cells than group B(p<0.05) showing bone damage caused by salt administration.Remarkable protection due to zinc was revealed by decrease in number of osteoclasts in group B whereas the mean difference between group C and B was statistically insignificant (p=0.286) (Table 4). When the slides were observed for number of osteoclasts in Femur, it was seen that group C had 2.466±1.060; group A had 4.333±0.975 and group B had 2.733±1.032 mean number of osteoclasts(Table 3). Group A had abundant osteoclasts (Fig 1); group B had scarce number of osteoclasts whereas group C showed more osteoblasts than group A and B .Intergroup comparison indicated the significant difference between groups C and A (p=0.000) and insignificant result between groups C and B. This confirmed the protective effect of zinc in group B (Table 4).The comparison of statistical results of histological parameters between group A and C showed that group A showed adverse changes due to high salt diet and group B exhibited reversal of changes.

Table 3: Mean Osteoclast number per unit area in humerus and femur of all groups

	Humerus			Femur		
Groups	C	A	B	C	A	B
Mean Score	2.666	4.400	2.600	2.46	4.333	2.733
Std. Deviation	1.046	0.985	1.242	1.06	0.975	1.032
SEM	0.270	0.254	0.320	0.27	0.251	0.266
p value	0.000*			0.000*		

Table 4: Comparison of mean osteoclast number per unit area in humerus and femur of all groups

	Humerus			Femur		
Groups	C vs. A	C vs. B	A vs. B	C vs. A	C vs. B	A vs. B
Mean Difference	-1.733	0.067	1.800	-1.866	-0.267	1.600
p value	0.000*	0.286	0.000*	0.000*	0.205	0.001*

Discussion

Decreased number of osteoblasts in group A clearly demonstrated the adversarial effects of high salt which are in agreement with other studies.[36,38] Decrease in Alkaline phosphatase activity and less new bone formation were suggestive of decrease osteoblast count. Less production of growth factors that regulate osteoblast differentiation has been documented in experimentally induced osteoporosis.[8]

Zinc promotes bone tissue formation by proliferation and differentiation in osteoblasts.[25,27,39] This can be due to protein synthesis as the concentration of osteocalcin, IGF-1 and TGF-β1 has been estimated to be increased in the osteoblast culture medium containing zinc. Diffusion of growth factors in osteoid and subsequent storage in bone takes place.[40] Bone mineralization property of zinc mediates through ALP activity and other metallo-enzymes. [28, 31] Increased cell proliferation in osteoblasts even at a low level of zinc, enhance ALP function and concentration of collagen in osteoblastic

MC3T3-E1 cells have been reported by further augmenting bone forming role of zinc. [22] Zinc stimulates the expression of runt-related transcription factor which is related to the differentiation of pre-osteoblastic cells.[40, 41]

Hard tissue formation takes place by zinc in the form of increased osteoblast and diminished osteoclast activity.[25, 27] In the present study, the protective role of zinc exhibited in the form of increase in number of osteoblast paralleled with a previous report .[27] Studies on the effect of zinc on bone components of rats and proposed that increase in osteocalcin production is responsible for bone mineralization and increased ALP activity.[42] Increased proliferation of osteoblastic cells is directly related to zinc ability to increase IGF-1 and ALP activity. [42] Brzoska also supported this by observing increase in the enzyme activity in cortical and trabecular bone. [29]

Bone loss is due to imbalance between its formation and resorption and moreover, nutritional factors induce bone resorption by increasing the number of osteoclasts, an important marker of bone resorption.[35,43] In the present study the number of osteoclasts was increased in high salt group A after eight weeks of research period. This is a confirmed fact by Ahmed who observed a significant increase in osteoclasts lining the irregular bone surface after administering salt to group of rats. [36] Govindarajan reported that increase bone resorption can be due to rise in osteoclasts number after hypocalcaemic induced secondary hyperparathyroidism in ovariectomized rats.[44] Matrix disorganization and atypical collagen deposition in the extracellular matrix may lead to scarce interaction between matrix and cells leading to imbalance between osteoblasts and osteoclasts and hence bone damage. [35]

Supplement intake with zinc suppresses osteoclastic bone damage and is a useful dietary regime for the avoidance and treatment of osteoporosis.[42] Zinc can inhibit bone resorbing factors like PTH, prostaglandin E2 and osteoclast formation by suppressing the enhancing effect of RANKL.[31] RANKL is a member of tumor necrosis factor (TNF) and is secreted from osteoblasts as a result of osteoporotic factors. Zinc by inhibiting the RANKL can decrease osteoclastogenesis.[45] It has been observed that osteoproteogerin (OPG) is also produced by osteoblasts which has the property of suppressing the activity of RANKL.[46] The inhibition of osteoclast-like cell formation by zinc may be due to stimulation of TGF-β or IGFwhich acts as a coupling factor with zinc.[31, 39, 40] These bone growth factors are involved in the DNA synthesis and therefore can increase bone components. [47] Hence, it can be inferred from on-going discussion that zinc supplementation can prevent the bone loss by decreasing osteoclastic activity.[26]

When culture of bone marrow cells having bone resorbing factors like PTH was observed, increased osteoclasts were demonstrated and after zinc addition, the number of osteoclasts was decreased. The reason may be due to the death of osteoclasts by apoptosis in the presence of zinc.[40]

Conclusion

1.Zinc supplementation can be considered an appropriate dietary strategy to reduce risk of osteoporosis.

2.Increase in bone forming cells and decrease in bone resorbing cells are observed after zinc administration to rats who were fed on high salt diet showing that zinc has protective role against high salt induced deleterious effects on bone cells of rats.

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