Biol Trace Elem Res (2017) 176:338–341
DOI 10.1007/s12011-016-0826-9

Effects of Zinc Supplementation on DNA Damage in Rats with Experimental Kidney Deficiency

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Received: 28 April 2016 / Accepted: 16 August 2016 / Published online: 10 September 2016
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Abstract This study was carried out to determine the effect of zinc on oxidative DNA damage in rats with experimental acute and chronic kidney deficiency. Six groups of five Wistar-Albino rats each were assigned as controls (C), acute kidney deficiency (AKD), zinc-supplemented (+Zn), acute kidney deficiency, zinc-supplemented (AKD + Zn), chronic kidney deficiency (CKD) and zinc-supplemented chronic kidney deficiency (CKD + Zn). The levels of 8-Oxo-2′-deoxyguanosine (8-OHdG) were determined, being the lowest in the CKD group ($p < 0.05$), higher in the C group than those of rats with CKD but lower than that of all the other groups ($p < 0.05$). There were no significant differences between the controls and the CKD + Zn group, or between the AKD and the +Zn groups. Among all groups, the highest 8-OHdG level was found in the AKD + Zn group ($p < 0.05$). DNA damage was greater in acute renal failure than in rats with chronic renal failure. The DNA damage in the zinc group was significantly higher than in the controls.

Keywords DNA damage · Renal impair · Zinc

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Introduction

Zinc is a trace element that is central to a number of metabolic processes that include gene expression, DNA synthesis, enzymatic catalysis, storage and release of hormones, neurotransmission, memory and sight, growth, and development. It has important roles in the metabolism of carbohydrates, proteins, lipids, and nucleic acids as well as in heme synthesis, growth, and embryogenesis. It is essential for the functions of enzymes that have roles on nucleic acid, carbohydrate, protein, and lipid metabolism. It also takes part in the functioning of carbonic anhydrase, alkaline phosphatase, alcohol dehydrogenase, carboxypeptidase, lactic dehydrogenase, glutamic dehydrogenase, aldolase, ribonuclease, DNA and RNA polymerases, and superoxide dismutase [1, 2].

Gentamicin is obtained from *Micromonospora purpurea,* a gram-positive microorganism that produces an aminoglycoside antibiotic capable to inhibit normal protein synthesis in some microorganisms [3]. Aminoglycoside-type and other antibiotics are commonly used for the treatment of urinary infections, but they have been reported as nephrotoxic [4].

Normally DNA is exposed to oxidative damage from endogenous and exogenous sources. 8-OHdG is a useful tool used as an indicator for oxidative stress and oxidative DNA damage. Among the four DNA bases, guanine and deoxyguanosine are the two DNA bases more susceptible to oxidation because of their lower ionization potential [5, 6].

The present study was planned to investigate the effect that zinc supplementation has on DNA damage in rats with experimentally induced kidney deficiency.

Materials and Methods

The Local Ethics Committee on Animal Experimentation of University of YuzuncuYıl in Van, Turkey, approved the experimental procedures used throughout this study.

Animals

For this study, 30 Wistar-Albino male rats weighing 200–250 g were divided into six groups of five rats. The rats were housed in cages under ordinary conditions with 12 h light/dark automatic lightning. Normal saline was injected for 3 days to prevent injection stress. After that, the study groups were treated as follows: control group, treated with daily IP injections of 0.5 cc normal saline.Acute kidney disease (AKD) group, treated with daily intraperitoneal injections of 100 mg/kg gentamicin sulfate every day for 8 days.Zinc-supplemented group (Zn+), the rats were given 227 mg/L zinc sulfate in their drinking water for four weeks and then continued through the duration of the study.Zinc-supplemented acute kidney deficiency group (AKD+Zn), in which the rats were supplemented as in the Zn+ group and then injected with gentamicin as described for group AKD.Chronic kidney deficiency group (CKD), injected with 50 mg/kg gentamicin sulfate injected intraperitoneally every day for 15 days.Zinc-supplemented chronic kidney deficiency group (CKD+Zn), rats supplemented with zinc four weeks before gentamicin injection as described for group CKD. Tables 1, 2, and 3 give the composition of food, minerals, and vitamins used in this study.

Determination of Serum 8-OHdG Levels

A DNA Damage EIA kit (Catalog No: ADI-EKS 3501, Enzo Life Science) was used to determine the levels of serum 8-OHdG. The plate absorbance was measured at 450 nm with an ELISA device (Anthus 20rt). A calibration curve was prepared using 8-OHdG standards and used to compare the 8-OHdG optical densities of the samples.

Table 1 AIN-76, American Institute of Nutrition Sample Diet [7]

Ingredients	kg/100 kg
Casein	20
dl-Methionine	0.3
Maize starch	15
Sucrose	50
Cellulose	5
Corn oil	5
Mineral mixture	3.5
Vitamin mixture	1
Choline bitartrate	0.2

Table 2 AIN-76, American Institute of Nutrition Vitamin Mixture Composition [7]

Vitamin mixture	Required for 1 kg
Thiamine-HCL	600 mg
Riboflavin	600 mg
Pyridoxine-HCL	700 mg
Nicotinic acid	3 g
D-calcium pantothenate	1.6 g
Folic acid	200 mg
D-Biotin	20 mg
Cyanocobalamin (vit B12)	1 mg
Retinol palmitate–acetate (vit A)	400.000 IU vit A
dl-α tocopherol acetate (vit E)	5.000 IU vit E
Colecalciferol (vit D)	2.5 mg

Statistical Analysis

The results were evaluated with a multiple comparison test. The values are given as averages ± SD for all groups. SPSS 22.0 statistical package program was used for statistical analysis.

Results and Discussion

8-OHdG is a byproduct of DNA damage. It consists of reactive oxygen and hydrogen species and is an indicator of oxidative stress. 8-OHdG is found in tissues, serum, urine, and other biological materials [8]. The 8-OHdG levels measured in this experiment are presented in Table 4. The lowest value was found in the CKD group ($p < 0.05$) and higher for the controls. It was in fact the lowest for all study groups. No

Table 3 AIN-76, American Institute of Nutrition Mineral Mixture Composition [7]

Mineral mixture	g/kg
Calcium phosphate dibasic ($CaHPO_4$)	500
Sodium chloride (NaCl)	74
Potassium citrate ($K_2C_6H_6O_7$. H_2O)	220
Potassium sulfate (K_2SO_4)	52
Magnesium oxide (MgO)	24
Manganese carbonate (43–44 % Mn)	3.5
Ferric citrate (16–17 % Fe)	6
Zinc carbonate (70 % Zn)	1.6
Copper carbonate (53–55 % Cu)	0.3
Potassium iodate (KIO_2)	0.01
Copper carbonate (53–55% Cu)	0.01
Chromium potassium sulfate	0.55
Sucrose to make up	1000 g

Table 4 Serum 8-OHdG levels in all study groups

Groups	8-OHdG (nmol/L)
Control	41.25 ± 407^{a}
AKD	57.08 ± 7.83^{c}
Zn+	52.14 ± 4.68^{c}
AKD + Zn	105.25 ± 9.45^{d}
CKD	29.04 ± 4.59^{b}
CKD + Zn	40.49 ± 6.52^{a}

Superscript letters represent statistical differences within the same column ($p < 0.05$)

significant difference was seen between the CKD + Zn and the controls or between the AKD and Zn + groups. The highest 8-OHdG level was seen in the AKD + Zn group ($p < 0.05$).

It has been reported that some chemicals can increase the levels of 8-OHdG. Increases of 8-OHdG level in the esophagus, liver, kidney, and lung were seen after experimental treatment with some chemicals like ethanol, 3-methylalcohol-4dimethylaminoazobenzene, ferrous nitrilotriacetate (FeNTA), and asbestos. It is thought that the formation of 8-OHdG in tissue DNA is one of the mechanisms of oxidative stress carcinogenesis [9].

The results here presented are in good agreement with those of various studies. Hunjoo Ha et al. measured the levels of 8-OHdG in the liver, pancreas, and kidney tissues on STZ-induced diabetes in rats and found a significant increase of 8-OHdG levels in diabetic kidney tissues relative to healthy controls [10]. Stoyanova et al. observed genetic damage increases along with the increase of renal function damage [11]. Lim et al. examined tissue damage and renal oxidative DNA through increased hemolysis by phenylhydrazine and observed a significant increase of the levels of renal DNA 8-OHdG [12]. Krivosíková et al. reported a number of DNA fractures in lymphocytes in kidney effluent increased in experimentally induced chronic kidney deficiency [13].

Hirotsu et al. found a significantly increased DNA damage in leucocytes and in the kidney, liver, and brain tissues in patients with chronic kidney impairment, proving the genotoxicity of this condition due to various uremic toxins and reactive oxygen radicals that result in oxidative DNA damage (p 0.05) [14]. It was observed that the levels of 8-OHdG were significantly increased in a group of nephropathy patients compared to healthy controls ($p < 0.05$) [15].

In this study, the DNA damage marker was significantly increased in all groups relative to controls except the CKD group ($p < 0.05$, Table 4). A significant increase of DNA damage marker in both acute- and chronic kidney deficiency groups can be regarded as an indicator of kidney tissue damage, which is in accordance with the literature given above. It is quite remarkable that the 8-OHdG levels in the CKD group were lower than those of the controls. It can be assumed that the blood concentration of 8-OHdG increased due to the initial damage to the kidney, which later decreases when urinary function stabilizes. Similarly, it was found that the level of 8-OHdG was unchanged between the control and CKD + Zn groups (Table 4).

Greater DNA damage was found in the AKD + Zn group. It is thought that kidney damage is higher as a result from zinc supplementation: DNA damage was increased in the AKD and Zn + groups, but were significantly lower in the AKD + Zn group, suggesting that Zn might induce ROS, genotoxicity, and oxidative DNA damage [16]. DNA damage was greater in the AKD group than in the CKD group. This situation indicated that 8-OHdG is removed from the bloodstream in the chronic case, and it can be useful in the follow-up of the metabolic consequence of 8-OHdG in future studies. Furthermore, since the DNA damage levels of all groups to which zinc was applied were observed to be high compared to the control, it was concluded that the high-Zn dose used in this study was a factor that resulted in increased oxidative DNA damage.

In conclusion, it was observed that in experimental acute kidney deficiency, additional Zn caused an increase of the 8-OHdG levels as a result of increased DNA damage to the kidney tissue. In chronic kidney deficiency, the level of this DNA damage marker dropped to a level that was below than in the control group.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest. All authors have read and approved the final manuscript.

References

1. Arcasoy A (2002) Çinko ve Çinko Eksikliği. Talasemi Derneği Yayınları, Ankara (In Turkish)
2. Rotsan EF, De Buys HV, Madey DL (2002) Evidence supporting zinc as an important antioxidant for skin. Int J of Dermatol 4:606–611
3. Anonymous (2009) Genta 160 mg I. E. ULAGAY. http://www.ilacabak.com/ilacgoster.php?Id=3384 Accessed 18 July 2009
4. Şahin T, Çamkerten İ (2003) Kedi ve köpeklerin böbrek hastalıklarında antibiyotiklerin kullanımı. YYU Vet Fak Derg 14(1):82–86 In Turkish
5. Greenberg MM (2004) In vitro and in vivo effects of oxidative damage to deoxyguanosine. Biochem Soc Trans 32: 46–50
6. Mc Dorman KS, Pachkowski BF, Nakamura J, Wolf DC, Swenberg JA (2005) Oxidative DNA damage from potassium bromate exposure in Long-Evans rats is not enhanced by a mixture of drinking water disinfection by-products. Chem Biol Interact 152:107–117
7. Reeves PG, Nielsen FH, Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of

Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 23:1939–1951
8. Shen J, Deininger P, Hunt JD, Zhao H (2007) 8-hydroxy-2-deoxyguanosine (8-OH-dG) as a potential survival biomarker in patients with nonsmall-cell lung canser. Cancer 109:574–580
9. Kasai H (2002) Serial review: oxidative DNA damage and repair: chemistry-based studies on oxidative DNA damage: formation, repair, and mutagenesis. Free Radic Biol Med 33:450–456
10. Ha H, Kim C, Son Y, Chung MH, Kim KH (1994) DNA damage in the kidneys of diabetic rats exhibiting microalbuminuria. Free Radic Biol Med 16:271–274
11. Stoyanova E, Sandoval SB, Zúňiga LA, El-Yamani NE, Coll S, Pastor J, Reyes E, Andrés J, Ballarin N, Xamena R (2010) Oxidative DNA damage in chronic renal failure patients. Nephrol Dial Transplant 25:879–885
12. Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH, Kastan MB (2000) ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. Nature 404:613–617
13. Krikosiva Z, Dusinska M, Spustova V, Sebekova K, Blazicek P, Heidland A, Dzurik R (2001) DNA damage of lymphocytes in experimental chronic renal failure: beneficial effects of losartan. Kidney Int 59:212–215
14. Hirotsu C, Tufis S, Riberio DA, Alvarenga TA, Andersen ML (2011) Genomic damage in the progression of chronic kidney disease in rats. Brain Behav Immun 25:416–422
15. Pan H, Zhang L, Guo M, Sui H, Li H, Wu W, Qu N, Liang M, Chang D (2010) The oxidative stress status in diabetes mellitus and diabetic nephropathy. Acta Diabetol 47:71–76
16. Annangi B, Rubio L, Alaraby M, Bach J, Marcos R, Hernández A (2015) Acute and long-term in vitro effects of zinc oxide nanoparticles. Arch Toxicol. doi:10.1007/s00204-015-1613-7