Role of Zinc on Antioxidative Enzymes and Lipid Peroxidation in Brain of Diabetic Rats

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Abstract

Background: Diabetes is the third leading cause of death in the United States after heart disease and cancer. It is a chronic and progressive illness that causes considerable morbidity and premature mortality. Research shows that zinc, an essential trace element responsible for over 300 enzyme functions, can aid in normalizing the negative effects of diabetes mellitus. Zinc plays an important role in metabolic pathways, and also regulates insulin production by pancreatic tissue and glucose utilization by muscle and fat cells. But not many studies have been performed regarding the antioxidative role of zinc on brain during diabetes.

Methods: Male Wistar rats were given zinc in the form of ZnSO4·7H2O at a dose level of 227 mg/L daily in their drinking water and diabetes was induced by giving a single intraperitoneal injection of alloxan (150 mg/kg body weight).

Results: The present study indicated that during diabetes, lipid peroxidation in brain was found to be increased while levels of GSH and the activity of catalase and SOD were found to be decreased. Zinc, however, was able to decrease lipid peroxidation and increase catalase activity in brain, but it further decreased the activity of GR and SOD.

Conclusion: Our findings suggest that oxidative stress occurs in diabetic state and that the oxidative damage to tissues may be a contributory factor in complications associated with diabetes. Zinc seems to positively influence lipid peroxidation and oxidative stress, but whether this effect is mediated by Cu, Zn- SOD needs to be explored further.

Keywords: Brain; Antioxidative enzymes; Lipid peroxidation; Diabetes; Zinc

Introduction

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism [1]. This disease is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The abnormality in insulin secretion and utilization causes glucose to accumulate in the blood, often leading to various complications [2]. Long term diabetes can lead to blindness, kidney failure, and nerve damage. Diabetes is also an important factor in accelerating the hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases. This is referred to as macro vascular disease.

During diabetes, there is disturbance in the levels of different metals like Zn, Cd, and Cu etc. Zinc plays an important role in insulin production in the β cells of islets of Langerhans. Zinc is an essential trace element required for a broad range of biological activities. It is non-toxic in physiological doses [3]. It is known to be associated with metal-binding proteins that regulate the functions of zinc as well as of copper [4]. Zinc is present in large amounts in the brain [5]. Zinc stabilizes the cell membrane structure through its antioxidative actions by regulating the levels of metallothionein [2], and has also been reported to inhibit spontaneous lipid peroxidation in the rate brain [6]. Thus, an abnormal zinc metabolism could play a role in pathogenesis of diabetes mellitus and some of its complications [5]. Moderate zinc deficiency may be relatively common in individuals with diabetes mellitus; increased loss of zinc by frequent urination appears to contribute to the marginal zinc nutritional status that has been observed in diabetes mellitus [7].

Increasing evidence in both experimental and clinical studies suggests that there is a close link between hyperglycemia, oxidative stress and diabetic complications i.e. there is overproduction of reactive oxygen species which affects virtually all cellular components, leading to DNA and protein modification and lipid peroxidation [8].

Antioxidant enzymes primarily account for intracellular defense, while several non-enzyme molecules, small molecular weight antioxidants, protect various components against oxidation in plasma. Intracellular antioxidant defense is primarily provided by antioxidant enzymes, which catalyze decomposition of reactive oxygen species. The three major antioxidant enzymes are Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase. It is maintained that high plasma lipid peroxides in diabetes may result from oxidative destruction of erythrocyte membrane lipids [9]. Glutathione acts as a direct scavenger as well as a co-substrate for GPx. Decrease in glutathione levels and increase in lipid peroxidation is a direct measure of increased oxidative stress. Elevated oxidative stress in diabetes plays an important role in the pathogenesis of diabetic complications [10].

Zinc is an essential trace mineral and the second most abundant in the brain following iron [11]. It is a component of almost every
living cell, including those of the muscles & bones (which contain about 90% of the body’s zinc), skin, liver, kidney, brain, blood, eye, pancreas and, in males, the prostate. The relationship between zinc and diabetes is complex and not fully understood. Studies on metal catalyst, like zinc, shows that it plays an antioxidant role. However, there is lack of information regarding the effect of zinc on the antioxidative enzymes in brain tissue during diabetes. So the present study was undertaken to study the status of antioxidants and lipid peroxidation in brain during diabetes and to evaluate the role of zinc under such conditions.

**Materials and Methods**

Wistar male rats (40) in the weight range of 120-160 g were obtained from the Central Animal House, Panjab University, Chandigarh. The animals were housed in polypropylene cages bedded with rice husk in the departmental animal house under hygienic conditions. All procedures were done in accordance with ethical guidelines for care and use of laboratory animals, and protocols were followed as approved by the Experimental Animals Committee.

The animals were divided in four main groups of 10 animals each:

- **Group I: Normal Control** – Animals in this group was given standard pellet feed and water *ad libitum* throughout the period of experimentation.

- **Group II: Diabetic** – Diabetes was induced in the rats using a single intravenous injection of Alloxan at the dose of 45 mg/kg body weight [12] in the beginning of the experiment.

- **Group III: Zinc treated** - Rats in this group were given zinc as was given to group III rats and diabetes was induced in a similar way as given to group 2 animals.

- **Group IV: Diabetes and Zinc treated** - Rats belonging to this group were given Zinc + Diabetes as compared to the group II. The levels were further reduced in group II (p<0.01), group III (p<0.05) as well as group IV (p<0.01) when compared with the group I

Animals in all groups were fed standard laboratory feed and water *ad libitum* throughout the period of experimentation. Diabetic status of rats was checked by urine reagent strips. Animals with urine glucose higher than 500 mg/dL at 3rd day of injection were considered diabetic. All the above treatments were given for a period of eight weeks. After experimentation, Diabetic status was checked by urine reagent strips. Animals with urine glucose higher than 500 mg/dL at 3rd day of injection were considered diabetic.

Lipid peroxidation was measured by the method of Hochstein et al. Since malondialdehyde is a degradation product of peroxidised lipids, the development of pink colour with the absorption maxima at 532 nm as a TBA-MDA chromophore has been taken as an index of lipid peroxidation. Reduced glutathione content was estimated in brain according to the method of Moron et al. [16]. Reduced glutathione is a non-protein sulphhydryl compound. 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) is a disulphide compound, which is readily reduced by sulphhydryl compounds (SH) to form 1 mole of 2-nitro-5-mercaptopbenzoic acid per mole of SH. The nitromercaptobenzoic acid anion released has an intense yellow colour and can be used to measure SH groups at 412 nm. Catalase activity was estimated by the method of Luck [17]. H$_2$O$_2$ was used as a substrate. The activity of superoxide dismutase was estimated according to the method of Kono [18]. The reduction of nitroblue tetrazolium (NBT) to blue formazan mediated by hydroxylamine hydrochloride was measured under aerobic conditions. Addition of SOD in homogenate inhibits the reduction of NBT by superoxide anions which were generated by photooxidation of hydroxylamine hydrochloride and extent of inhibition was taken as measure of enzyme activity. Glutathione reductase was assayed by the method of Williams and Arscott [19]. Glutathione reductase is a flavoprotein that catalyzes the NADPH- dependent reduction of glutathione disulphide (GSSG) to GSH. The amount of protein contents in brain were determined by the method of Lowry et al. [20]. The method is based on colour reactions of amino acids namely tryptophan and tyrosine with the folin phenol reagent. The protein in the sample was treated with alkaline copper tartarate to form cupric amino acid complex. The intense blue colour was formed due to the reduction of phosphomolybdic acid and phosphotungstic acid by aromatic amino acids (tyrosine and tryptophan) and cupric amino acid complexes.

**Statistical analysis**

Tabulated values represent means ± S.D. Student’s t-test was used to analyze the data from experimental and control groups. Values of p<0.05 were considered as significant.

**Results**

The present study indicated a significant increase (p<0.05) in lipid peroxidation in group II animals as compared to the group I. However, lipid peroxidation was found to be decreased in all the treatment groups when compared with group I but this decrease was not statistically significant. Lipid peroxidation in group IV animals were found to be further decreased significantly (p<0.01) when compared with the group IV animals (Table 1, Figure 1).

Glutathione levels were found to be decreased significantly in brain of group II (p<0.01), group III (p<0.05) as well as group IV (p<0.01) as compared to the group I rats and the levels were further reduced in group IV rats when comparison was made with the group II (Table 2, Figure 2).

**Table 1: Effect of zinc on lipid peroxidation in brain of diabetic rats.**

| Groups                  | Lipid peroxidation (nmol MDA formed/mg protein) |
|-------------------------|-----------------------------------------------|
| I Control               | 1.16±0.23                                     |
| II Diabetic             | 1.26±0.24*                                    |
| III Zinc                | 0.90±0.16                                     |
| IV Zinc + Diabetic      | 0.77±0.04*                                    |

Values are expressed as Mean± S.D

*p<0.05 when the values of group II are compared with those of group I

#p<0.01 when the values of group IV are compared with those of group II

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The activity of catalase was found to be decreased significantly (p<0.01) in group II rats as compared to the group I ones. However, the catalase activity was found to be increased significantly in group IV as compared to the group I (p<0.01) and group II rats (p<0.001) (Table 3, Figure 3). Catalase activity was not found to be altered significantly in group III rats as compared to the group I.

Superoxide dismutase activity was found to be significantly decreased (p<0.05) in group II, in group III rats (p<0.05) and group IV rats (p<0.01) as compared to the group I rats and the activity was further reduced (p<0.05) in group IV rats (Table 4, Figure 4).

Glutathione reductase activity was found to be significantly raised (p<0.001) in group II rats as compared to group I. On the other hand, the enzyme activity was found to be reduced significantly (p<0.01) in group IV rats as compared to the group I rats and the activity was further reduced (p<0.05) in group IV rats (Table 4, Figure 4).

The activity of catalase was found to be decreased significantly (p<0.01) in group II rats as compared to the group I ones. However, the catalase activity was found to be increased significantly in group IV as compared to the group I (p<0.01) and group II rats (p<0.001) (Table 3, Figure 3). Catalase activity was not found to be altered significantly in group III rats as compared to the group I.

Superoxide dismutase activity was found to be significantly decreased (p<0.05) in group II, in group III rats (p<0.05) and group IV rats (p<0.01) as compared to the group I rats and the activity was further reduced (p<0.05) in group IV rats (Table 4, Figure 4).

Glutathione reductase activity was found to be significantly raised (p<0.001) in group II rats as compared to group I. On the other hand, the enzyme activity was found to be reduced significantly (p<0.01) in group IV rats as compared to the group I and also when compared with the group II rats (Table 5, Figure 5). Here again group III animals did not show any significant change in the enzyme activity as compared to group I.

**Discussion**

As evident by the results, increase in lipid peroxidation and decrease in glutathione and the activity of SOD and catalase indicate the existence of the oxidative stress in diabetic rats. Diabetes has been reported to induce alterations in the antioxidative defence capacity in brain, which could result in increased risk of peroxidative damage. This increase in lipid peroxidation during diabetes is due to peroxidation of polyunsaturated fatty acids, leading to the degradation of phospholipids, which is considered as an index of cellular deterioration [21]. Studies have shown that zinc causes inhibition of both endogenous as well as induced lipid peroxidation to stabilize biomembranes [22]. Also there are reports of zinc deficiency during diabetes and it has been suggested that peroxidation of the membrane phospholipids occurs more readily in cells with low zinc status [23]. The present study showed that zinc counteracts the lipid peroxidation produced by diabetic conditions. This would appear to be due to zinc’s antiperoxidative properties. Zinc is known to protect fatty acids from peroxidation by inhibiting the production of reactive oxygen species [24]. Decrease in lipid peroxidation in zinc treated rats suggests the antioxidative effect of zinc on brain as described by other workers also [14]. Similar property of zinc is also responsible for decrease in
lipid peroxidation observed in combined zinc and diabetic rats. Zinc treatment may overcome, to some extent, the excessive formation of free radicals which are induced during diabetes and thus leads to decrease in lipid peroxidation in brain. The protection imparted by zinc may, in part, also be mediated by the activation of metallothionein synthesis [25]. These zinc metallothionein antagonizes in inhibiting lipid peroxidation in the brain.

Glutathione offers one of the several mechanisms for the scavenging of toxic free radicals [26]. Glutathione helps preserve brain tissue by preventing damage from free radicals. The decrease in glutathione levels during diabetes could be due to enhanced oxidation of glutathione to GSSG which is due to increased generation of free radicals leading to an oxidative stress during diabetes. This decrease is concomitant with an enhancement of lipoperoxidative processes [27]. Glutathione binds to foreign compounds and their metabolites excreted resulting in decreased concentration of this tripeptide following the formation of free radicals during diabetes. These enzymes might be affected by zinc intake might be due to stimulation of the system which is affecting enzymes at the gene level by unexplained mechanism. Further studies are required to study such mechanism and to confirm the antioxidative role of zinc in brain.

The observed decrease in catalase activity during diabetes is the first detectable effect on the protective set of enzymes against oxygen toxicity. This may lead to perturbation in the antioxidative defence. In our study, we found significant decrease in the activity of catalase in brain of diabetic rats which is attributed to the increased stress conditions and loss of micronutrients that prevents the normal functioning of the enzyme. On the contrary, combined zinc and diabetic rats showed a significant rise in catalase activity which might be due to the induction of zinc metallothionein, which are also involved in antioxidant defense system [30].

The activity of superoxide dismutase was also found to be decreased during diabetes. This might be due to the fact that there is excessive formation of free radicals during diabetes. These enzymes might be used up in scavenging these radicals, thus leading to their decreased activity during diabetes. Also zinc deficiency has been reported to be associated with diabetes which might lead to decrease in the activity of superoxide dismutase and thus causes further accumulation of free radicals. The balance between antioxidant enzymes, superoxide dismutase and catalase is important for cell function [31]. However, in the present study, the antioxidant balance in the brain has been altered during diabetes which may perturb the brain cell normal functioning. Both zinc treatment and combined zinc and diabetes group showed a decrease in the activity of enzymes which might be due to the fact that copper levels were the controlling factors rather than zinc concentration. Yang and Cherian have also demonstrated that STZ-induced lipid peroxidation can be improved with zinc without any changes in SOD activity [32]. It is possible that 8 weeks of study has caused an enormous increase in free radicals. Pigeolet et al. [33] has shown that SOD can also be inactivated by the products of their own reactions in the presence of high concentrations of H$_2$O$_2$ or hydroxyl radicals. Increase in the activity of glutathione reductase during diabetes represents an adaptive change against lipid peroxide toxicity [27].

In conclusion, data from the present study showed that zinc is not playing any protective role in diabetes in brain tissue and the observed changes due to zinc intake might be due to stimulation of the system which is affecting enzymes at the gene level by unexplained mechanism. Further studies are required to study such mechanism and to confirm the antioxidative role of zinc in brain.

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