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Zinc and the diabetic heart

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Abstract

Zinc (Zn) is an essential mineral that is required for various cellular functions. Its abnormal metabolism is related to certain disorders such as diabetic complications. Oxidative stress has been considered as the major causative factor for diabetic cardiomyopathy. Zn has a critical antioxidant action in protecting the heart from various oxidative stresses. Zn deficiency was found to be a risk factor for cardiac oxidative damage and supplementation with Zn provides a significant prevention of oxidative damage to the heart. Diabetes causes a significant systemic oxidative stress and also often is accompanied by Zn deficiency that increases the susceptibility of the heart to oxidative damage. Therefore, there is a strong rationale to consider the strategy of Zn supplementation to prevent or delay diabetic cardiomyopathy. This short article collects the preliminary evidence, based on our own studies and those by others, for a preventive effect of Zn supplementation on diabetes-induced injury to the heart in animals and under in vitro conditions. Possible mechanisms by which Zn supplementation prevents diabetic heart disease are discussed. They include an antioxidant action of Zn, insulin function and metallothionein induction. In the final section, the future of Zn supplementation for diabetic patients is also briefly discussed. Although Zn supplementation has not been clinically used to prevent diabetic complications, because several issues need to be addressed, the fact that Zn supplementation is being used clinically for other disorders encourages us to explore its direct clinical application for the prevention of diabetic cardiomyopathy.

Abbreviations: Cu – copper; eNOS – endothelial NOS; Gpx – glutathione peroxidase; GSH – glutathione; HG – high levels of glucose; MT – metallothionein; MTT assay – a cytotoxic assay by measuring the reduction of tetrazolium compound into an insoluble formazan product; NO – nitric oxide; NOS – NO synthase; ROS – reactive oxygen species; SOD – superoxide dismutase; Tg – triglyceride; ZIP – Zrt-,Irt-like protein; Zn – zinc; ZnT – Zn transporter.

Introduction

Zinc (Zn) as an essential metal, is an important nutrient, cofactor of numerous enzymes and transcription factors (Coleman 1992; Vallee & Falchuk 1993; Cai *et al.* 2005). It is a constituent of more than 300 catalytically active Zn metalloproteins and more than 2000 Zn dependent transcription factors. Zn participates in a wide variety of metabolic processes including carbohydrate, lipid, protein and nucleic acid synthesis or degradation. Zn homeostasis results from a coordinated regulation by different proteins involved in uptake, excretion and intracellular storage/trafficking of Zn. These proteins are membranous transporters, belonging to the ZIP (Zrt-, Irt-like protein) and ZnT (Zn transporter) families, and metallothioneins (MT) (Palmiter & Findley 1995; Gaither & Eide

2001; Kambe *et al*. 2004; Palmiter 2004). Abnormalities of Zn homeostasis such as its deficiency may be associated with various pathogeneses of chronic diseases.

Diabetes mellitus is a major risk factor for early onset of coronary heart disease. In Type 1 diabetes there is a lack of insulin production and in Type 2 diabetes resistance to the effects of insulin is predominant. Both Type 1 and Type 2 have the same long-term complications including cardiomyopathy, retinopathy, nephropathy and neuropathy (Bardsley & Want 2004). Currently, treatment involves diet modification, weight reduction, exercise, oral medications, and insulin, but there is still much to learn about this disease. Recent insights into the mechanisms for diabetes onset and its complications strongly attracted the attention to alternative medicine such as Chinese natural medicines including minerals for treatment of diabetes and its complications (Salgueiro *et al*. 2001; Li *et al*. 2004).

The present article aims to summarize the information from the literature and our own studies, especially on the issues: (1) Effect of Zn status on the heart; (2) Zn status in diabetic subjects; (3) The evidence that, and possible mechanisms how Zn prevents diabetic cardiotoxicity. In the final section, the future of Zn therapy for diabetic cardiomyopathy is briefly discussed.

Effect of zinc status on the heart

Zinc deficiency is a risk for cardiomyopathy

Contractile function of the heart dictates its high metabolic demand, and the mitochondrial respiratory chain is the primary energy-releasing system in these cells. Mitochondrial respiration takes place in every cell and reactive oxygen species (ROS) are continually formed. Therefore, an efficient antioxidant system including superoxide dismutase (SOD), catalase, glutathione peroxidase (Gpx), glutathione (GSH), and α-tocopherol would be critical to the health of the heart. However, in experimental animal models, the activities of all of these antioxidants in the heart are low as compared to liver under normal (Doroshow *et al*. 1980) or adriamycin challenging conditions (Chen *et al*. 1994). The low antioxidant capacity makes the heart more susceptible to oxidative damage.

As Zn is a potent antioxidant (Bray & Bettger 1990; Prasad *et al*. 2004), Zn deficiency is a cause for the increased oxidative damage in multiple organs including the heart (Oteiza *et al*. 1995; 2000; Noh & Koo 2001; Ho & Ames 2002), due to the decreased cardiac antioxidant capacity (Kim & Keen 1999; Noh & Koo 2001). In addition, Zn deficiency exacerbates hypertension in hypertensive rats (Sato *et al*. 2002) and it is discussed as a risk factor for atherosclerosis (Anetor *et al*. 2002; Beattie & Kwun 2004). Both hypertension and atherosclerosis directly initiate or accelerate ischemic damage to the heart.

Zinc supplementation prevents cardiac toxicity from non-diabetic oxidative stress

Zn is part of an important adaptive function when tissue is challenged by oxidative stress. Cardiac adaptation to oxidative stress temporally redistributes systemic Zn into the heart, leading to low serum Zn and increased cardiac Zn levels at the early stage of cardiac pathogenesis (Yarom *et al*. 1977; Ripa *et al*. 1998; Norishima *et al*. 1999). In support of an antioxidant action of Zn in the heart, Zn supplementation indeed significantly protects the heart against the oxidative damage by a variety of oxidative stresses, including isoproterenol (Chvapil & Owen 1977), catecholamine (Singal *et al*. 1981), vitamin D3 (Wrzolek 1985), ischemia or/and perfusion (Powell *et al*. 1994, 1997) and adriamycin (Satoh *et al*. 2000; Ali *et al*. 2002).

Zinc status in diabetic subjects

Epidemiological studies have demonstrated that exposure to low concentrations of Zn in drinking water is associated with an increase in type 1 diabetes (Haglund *et al*. 1996; Zhao *et al*. 2001). Studies using animal models showed that various Zn chelators induce diabetes in some mammalian species, e.g. rabbits, mice, and hamsters, by β-cell destruction (Goldberg *et al*. 1990, 1991), and that Zn deficiency significantly enhances the blood glucose level in diabetes-prone experimental animals (Kechrid *et al*. 2001). Therefore, Zn deficiency can be considered a risk factor for the development of diabetes.

Diabetes also significantly impairs Zn homeostasis. Terres-Martos *et al*. (1998) examined the status of serum Zn in 18 patients with diabetes and

compared it to healthy, age matched controls. Serum Zn concentrations were significantly lower in diabetic patients than those in controls. In contrast, copper (Cu) levels were not significantly different from those in controls. These findings were supported by later studies (Anderson *et al*. 2001; Anetor *et al*. 2002; Roussel *et al*. 2003).

Several studies, therefore, have examined whether Zn supplementation prevents systemic Zn deficiency and other general parameters of diabetic subjects. Two clinical studies found an improvement of systemic Zn and antioxidant status (Faure *et al*. 1995; Kajanachumol *et al*. 1995). Zn supplementation significantly enhanced diabetes-decreased serum Zn level and antioxidant contents in blood cells. These studies were supported by subsequent clinical and animal studies (Gupta *et al*. 1998; Yamaguchi & Uchiyama 2003; Uchiyama & Yamaguchi 2003). Therefore, all these observations strongly support the notion that Zn deficiency occurs in diabetic subjects and that Zn supplementation will benefit or correct the diabetes-induced Zn status and systemic antioxidant capacity.

Zinc and the diabetic heart

Experimental evidence for protective effects of Zn on the diabetic heart

Based on the above discussion, diabetes is associated with Zn deficiency and Zn deficiency is also a risk factor for cardiac oxidative damage. Zn supplementation provides significant protection of the heart from oxidative stresses. Whether Zn supplementation prevents diabetic cardiomyopathy has not been addressed before, but clinical (Gupta *et al*. 1998) and animal studies (Yamaguchi & Uchiyama 2003; Uchiyama & Yamaguchi 2003) indicate that Zn supplementation provides a significant improvement of other diabetic complications such as the severity of peripheral neuropathy in diabetic patients and bone loss in diabetic animals. Therefore, we can assume that Zn supplementation may also improve cardiac dysfunction or damage in diabetic subjects.

To date, however, few studies have been performed on this specific issue. Mooradian *et al*. (1988) have investigated the role of Zn status in altered cardiac adenylate cyclase activity in diabetic rats. To determine whether the Zn intake of the animals can account for the altered beta-adrenergic receptor activity in the diabetic heart, the beta-adrenergic receptor number and isoproterenol-, fluoride- and forskolin-stimulated adenylate cyclase activity were examined in diabetic and control rats maintained on low, normal and high Zn diets for three weeks. Isoproterenol-stimulated adenylate cyclase activity was significantly lower in diabetic rats on low Zn diets compared with those on a control diet. Fluoride- and forskolin-stimulated adenylate cyclase activities were not significantly altered in diabetic rats. Low dietary Zn intake compared with high Zn diet significantly increased fluoride- and forskolin-stimulated adenylate cyclase activity in both diabetic and control rats. This study indicates that Zn intake appears to be an important determinant of cardiac adenylate cyclase activity level.

Recently, we demonstrated that Zn supplementation of STZ-induced diabetic mice for 3 months significantly prevented the development of cardiomyopathy, evaluated by morphological and functional examination at 6 months after diabetes, when compared to non-Zn-treated diabetic mice (Song *et al*. 2004). We also examined cardiac (H9c2) cells exposed to conditions that mimic those in diabetes: high levels of glucose and triglyceride (HG/Tg). As shown in Figure 1a,b, neither HG nor Tg (except for the highest dose of 50 μmol/l) alone caused significant cytotoxicity, evaluated by MTT assay (see the legend of Figure 1 for MTT assay). When cells were exposed to a combination of HG (22.5 mmol/l) and Tg, however, a significant cytotoxic effect was observed (Figure 1b). When these cells were pretreated with Zn (50 μmol/l) for 24 h, significant prevention of Tg- or HG/Tg-induced cytotoxicity was noted (Figure 1c,d). These results suggest that Zn protects against diabetes- or HG/Tg-induced cardiac toxicity *in vivo* and *in vitro*.

Possible mechanisms of Zn prevention of diabetic cardiomyopathy

As discussed above, Zn acts as a potent antioxidant. Therefore, the antioxidant action of Zn could be considered as the first possible mechanism. Zn functions as a complex antioxidant through participation in SOD and thioredoxin enzymatic and chelator activities, stabilizing cell

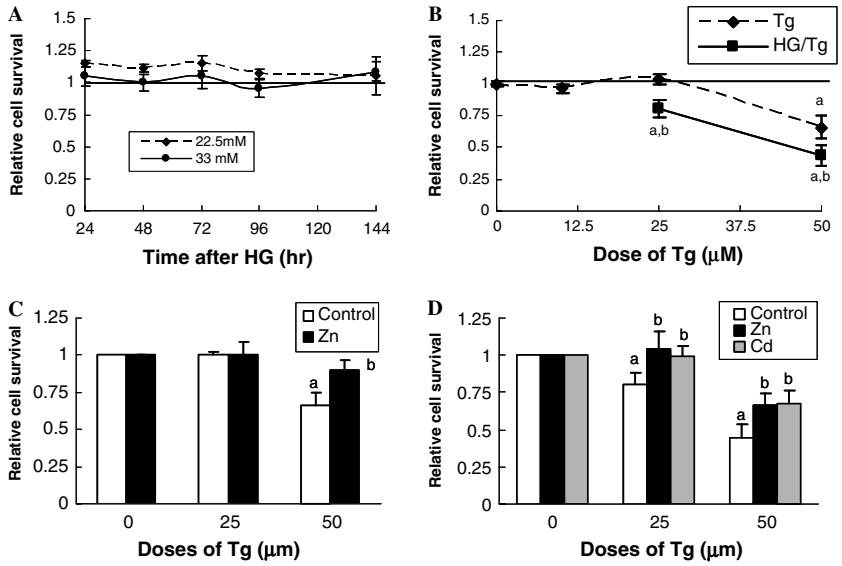

Figure 1. Tg-induced cytotoxicity with and without HG and Zn or Cd prevention. Cardiac (H9c2) cells were exposed to high levels of glucose (HG: 22.5 mM or 33 mM, panel A) for different times, triglyceride (Tg) at different concentrations (dash line in the panel B), HG (22.5 mM) + Tg (25 or 50 μM, solid line in the panel B), Tg (50 μM) with and without Zn (panel C), or HG/Tg (22.5 mM HG/25 or 50 μM Tg) with and without Zn or Cd (panel D) for 24 h, and then the cell viability was measured by MTT assay. Zn (50 μM, Black bars in panel C and D) or Cd (1.0 μM, Grey bars in panel D) was added into medium for 24 h before cells were exposed to Tg (White bars in panel C) or HG/Tg (white bars in panel D). a, $p < 0.05$ *versus* control (i.e. the survival rate of cells without any treatment), which is indicated, as 1, by the horizontal line crossing at 1 of Y axis in panes A and B, and indicated by white bars (Tg = 0) in panels C and D; b, $p < 0.05$ *versus* corresponding Tg alone group in panel B (indicated by diamond symbols with dash line) or HG/Tg in panes C and D. **MTT** assay is a colorimetric assay system which measures the reduction of a tetrazolium compound (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent for 4 h, a detergent solution is added to lyse the cells and solubilize the colored crystals. The samples are read using an ELISA plate reader at a wavelength of 540 nm. The amount of color produced is directly proportional to the number of viable cells.

membranes, and inhibiting lipid peroxidation (Collet *et al.* 2003; Prasad *et al.* 2004). In addition to the antioxidant action, the following actions of Zn are also important for the preventive effect.

Critical component of Zn in certain key enzymes and proteins: Since Zn is a critical factor for many important proteins, enzymes, and transcription factors, Zn deficiency may cause these enzymes' or proteins' dysfunction, leading to initiation or acceleration of cardiac pathogenesis in the diabetic subjects. Nitric oxide (NO) is produced by NO synthase (NOS) in many cells and plays important roles in the neuronal, muscular, cardiovascular, and immune systems, for which Zn is required (Raman *et al.* 1998). Diabetes causes overproduction of peroxynitrite through overproduction of superoxide (Brownlee 2001; Cai & Kang 2003). Peroxynitrite as a strong oxidant is capable of releasing Zn from the Zn-thiolate cluster of endothelial NOS (eNOS) leading to dysfunction of eNOS catalytic activity, i.e. decreases NO synthesis and increases superoxide production, called eNOS uncoupling (Zou *et al.* 2002). The eNOS uncoupling was found to play an important contribution to various cardiovascular diseases (Cai *et al.* 2003; Dixon *et al.* 2003; Zou *et al.* 2004). Zn deficiency is accompanied by an increase in oxidative stress (Oteiza *et al.* 1995, 2000; Noh & Koo 2001), and may accelerate the peroxynitrite-induced eNOS uncoupling. There is work indicating that Zn deficiency constitutes a pathogenic risk for the cardiovascular system (Mooradian *et al.* 1988). The fact that Zn deficiency exacerbates diabetic osteoporosis (Fushimi *et al.* 1993) supports the importance of Zn in maintaining the bone's normal function, and also supports the notion that Zn is important in maintaining the heart's normal function (Ripa *et al.* 1998).

Insulin function: In Type 1 and type 2 diabetes, either not enough insulin is produced or peripheral tissues have insulin resistance. The complications of diabetes are attributed either to the toxic effect

of hyperglycemia on target tissues or to abnormal metabolism of target tissues due to the insulin defect or resistance. Zn deficiency was found to decrease the response of tissues to insulin (Faure *et al*. 1992; Miranda *et al*. 2004), suggesting an insulin-like function. Indeed, Zn was found to decrease whole blood glucose levels in db/db diabetic mice (Simon *et al*. 2001) and increase leptin production (Mantzoros *et al*. 1998; Chen *et al*. 2000). Supplementation with Zn to diabetic patients may thus stimulate tissues to use glucose and maintain normal lipid metabolism to avoid the oxidative stress derived from the abnormal glucose and lipid metabolism.

Metallothionein (MT) induction: MTs are a group of intracellular metal-binding proteins, with high cysteine content (30% of all constituent amino acids). MTs are not only able to prevent the acute toxicity of heavy metals by their metal-binding capacity, but also capable of protecting cells or tissues from oxidative damage (Cai *et al*. 1999; Kang 1999). MTs are extraordinarily efficient in scavenging free radicals, particularly the hydroxyl free radical and hydrogen peroxide as compared to other antioxidants such as GSH. MT as a potent antioxidant protects cells and tissues from oxidative damage. Zn as a potent MT inducer protects these cells and tissues including the heart from oxidative stress-induced damage (Kang 1999; Cai *et al*. 1999; Satoh *et al*. 2000; Ali *et al*. 2002).

Since diabetic cardiomyopathy is directly related to oxidative stress and MT is a potent antioxidant, we and others have used an MT-transgenic (MT-TG) mouse model, in which MT mainly binds with Zn, to demonstrate that MT-TG mice are highly resistant to diabetes-induced damage to the heart (Cai & Kang 2001b; Liang *et al*. 2002; Cai *et al*. 2003). These results suggest that MT induction may be one of the mechanisms for the preventive effect of Zn supplementation on cardiomyopathy. Our recent pilot study (Song *et al*. 2004) demonstrated that supplementing diabetic mice with Zn significantly prevents the development of diabetic cardiomyopathy, along with significant induction of cardiac MT synthesis. In the *in vitro* study (Wang *et al*. 2004), exposure of cardiac cells to HG/Tg causes significant cytotoxicity, but this cytotoxicity was significantly inhibited by pre-treatment not only with Zn but also with low-dose cadmium (Cd) (Figure 1e). Cd is a toxic metal and also a potent inducer of MT (Onosaka & Cherian 1981). We showed that pre-treatment with low-dose Cd significantly induced MT synthesis in neuronal tissues and cells, leading to a significant protection from ionizing radiation (Cai *et al*. 2000, 2004). Therefore, the significant protection of cardiac cells from HG/Tg by pre-treatment with low-dose Cd is most likely due to MT induction (Wang *et al*. 2004). Taken together, these results strongly suggest that cardiac MT induction may play an important role in the preventive effect of Zn supplementation on diabetic cardiomyopathy.

Conclusion

There were several pieces of evidence indicating that Zn supplementation seems beneficial for the patients with diabetes to control glucose levels, correct lipid metabolism and prevent cardiovascular complications; however whether this means that Zn supplementation can immediately be used to treat or prevent diabetic cardiomyopathy remains to be determined (Salgueiro *et al*. 2001). In general, Zn is not very toxic, but excessive and chronic Zn supplementation still causes several symptoms including Cu deficiency (Cai *et al*. 2005), which is also a risk factor for cardiomyopathy (Davison *et al*. 1992; Nath 1997). Therefore, Zn supplementation together with other components such as chromium, magnesium, low-dose Cu and antioxidants has been proposed (DiSilvestro 2000; Anetor *et al*. 2002; Baydas *et al*. 2002; Bonham *et al*. 2003; Alissa *et al*. 2004). In addition, Zn salts have several disadvantages such as low absorption and short turnover. Zn complexes with superior properties that will overcome these limitations are being developed (Kojima *et al*. 2003; Song *et al*. 2003; Yoshikawa *et al*. 2004). A promising future for Zn supplementation will warrant further studies.

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