Diabetes and Metallothionein

Xiaokun Li[1], Lu Cai[1,2,*] and Wenke Feng[2]

[1]*Chinese-American Research Institute for Diabetic Complications, School of Pharmaceutical Sciences, The Wenzhou Medical College, Wenzhou, Zhejiang 325035, PR China;* [2]*Department of Medicine, University of Louisville School of Medicine, Louisville, KY 40202, USA*

Abstract: Diabetes is a widespread disease, and its development and toxic effects on various organs have been attributed to increased oxidative stress. Metallothionein (MT) is a group of intracellular metal-binding and cysteine-rich proteins, being highly inducible in many tissues. Although it mainly acts as a regulator of metal homeostasis such as zinc and copper in tissues, MT was found to be a potent antioxidant and adaptive (or stress) protein to protect cells and tissues from oxidative stress. Studies showed that zinc-induced or genetically enhanced MT synthesis in the pancreas prevented the development of spontaneous or chemically-induced diabetes. Genetically or pharmacologically enhanced MT expression in various organs including heart and kidney provided significant protection from diabetes-induced organ dysfunction such as cardiomyopathy and nephropathy. These studies suggest that MT as an adaptive protein can prevent both diabetes development and diabetic complications. This mini-review will thus briefly describe MT's biochemical features and then summarize the data on the protective effect of MT against diabetes and diabetic complications. In addition, the coordinative role of MT with zinc in the prevention of diabetes and its complications will also be discussed.

Key Words: Diabetes, metallothionein, antioxidant, diabetic complications, insulin-like action.

INTRODUCTION

Diabetes affects many Americans and other populations around the world. Cardiovascular and neuronal injury from diabetes contributes greatly to various diabetic complications including nephropathy, cardiomyopathy, retinopathy and neuropathy and even wound healing problems [1,2]. Several mechanisms have been proposed which are suggested to cause the development of diabetes and its complications. However, over-generation of oxidative stress has been considered to be a major contributor to the pathogenesis. Developing a strategy for the prevention of diabetic onset and its complications through the suppression of oxidative stress has thus received investigative attention [1-3]. A pioneered study by Dr. Cherian has demonstrated that pancreatic synthesis of metallothionein (MT), a potent antioxidant, induced by zinc (Zn) supplementation significantly prevented chemical streptozotocin (STZ)-induced diabetes [4]. We have primarily shown that overexpressed MT in the mouse heart prevented the manifestation of diabetes-induced cardiomyopathy [5,6]. In this article, therefore, we will summarize these studies on the prevention of diabetes onset and diabetic complications to extend our insights into MT prevention of diabetes and its toxicity.

MT AND ITS BIOCHEMICAL FEATURES

MTs have unique structural characteristics. They constitute a superfamily of nonenzymatic polypeptides consisting of 61-68 amino acids [7]. In mammals, four major subtypes of MTs have been identified. MT-III has been described as a neuronal regulator due to its major distribution in the brain [8]. MT-I and MT-II are expressed ubiquitously in mammalian tissues and are regulated and produced coordinately. They are often considered an entity in functional aspect.

One of the unique properties of MTs is its peculiar amino acid composition, characterized by the absence of aromatic amino acids or histidine and the high cysteine content. MT-I and MT-II consist of 61 and 62 amino acids respectively, of which 20 are cysteine residues. The polypeptide chain arranges cysteines in series of motifs: C-X-C, C-X-C-C, C-X-X-C, which are conserved across species. This distribution facilitates the formation of tetrathiolate clusters that confer on MT a highly stable tertiary structure capable of binding heavy metals with high affinity.

The cysteine sulfhydryl groups in MT bind and coordinate 7 Zn(II) and cadmium [Cd(II)] and 12 copper [Cu(I)] and silver [Ag(I)] [9]. The binding of divalent metals to MT occurs in two dumbbell shaped domains, of which the N-terminal β-domain usually binds 3 metals, whereas the C-terminal α-domain binds 4 metals (Fig. **1**). Under physiological conditions, MT primarily binds Zn, but the bound Zn can be replaced by other metals such as Cu, Cd and iron (Fe) under certain stress conditions [9,10].

The high metal binding capacity confers MT a pivotal role in metal manipulation, such as essential metal homeostasis and heavy metal detoxification [11-13]. Pharmacological and genetic studies in recent years have shown that MT has a protective function against oxidative injury [14,15]. Although the sulfhydryl residues of cysteine become firmly complexed upon metal binding, studies have shown that the thiol groups remain reactive under oxidative or nitrosative stress conditions [16,17]. In addition, when MT is exposed to an excess of dithiodipyridine, all 20 of its cysteines are oxidized within 1 hr with the concomitant release of all 7 Zn atoms [18]. Also, reaction of MT with 5,5'-dithiobis-2-nitro-

*Address correspondence to this author at Department of Medicine, University of Louisville School of Medicine, 511 South Floyd Street, MDR 533, Louisville, KY 40202, USA; Tel: 502-852-5215; Fax: 502-852-6904; E-mail: l0cai001@louisville.edu

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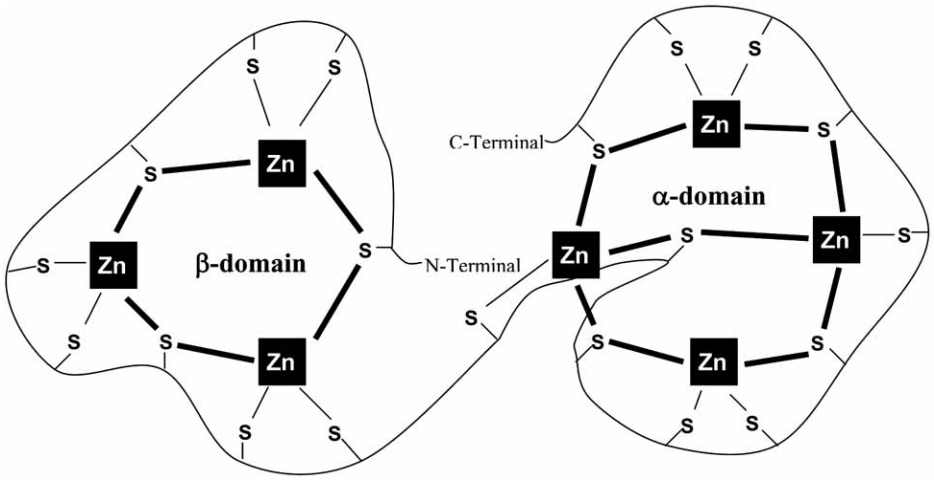

Fig. (1). Schematic illustration of the mammalian MT-II protein. There are 20 cysteine residues distributed in two metal-thiolate domains (α- and β-domains). Their sulfur atoms are able to bind monovalent or divalent metals (in this case 7 Zn). α-domain usually binds four divalent or six monovalent ions, whereas β-domain is capable to bind three divalent or six monovalent ions. The cysteine residues are either bridging, which is able to bind two divalent ions, or terminal, which binds only one divalent ion.

benzoic acid caused the formation of both intra- and intermolecular Cys-Cys disulfides and a small number of mixed disulfides [19].

Attempts to demonstrate the aforementioned formation of MT disulfide bonds *in vivo*, which may be responsible for the role of MT as an antioxidant, were impracticable in the past largely because of insufficient MT concentration in the tissue. Recently, we have used a cardiac MT transgenic mouse model and demonstrated the presence of MT disulfide bond formation in the hearts of MT-TG mice. More disulfide bonds were found under oxidative stress conditions induced by an antineoplastic agent doxorubicin [20].

The concomitant release of Zn from MT under oxidative stress conditions has been demonstrated in cultured cells and cell-free systems [18,21,22]. Under physiological conditions, the formation of MT disulfide bonds could be involved in the regulation of Zn homeostasis *via* Zn release from MT. Additional Zn release from MT under oxidative stress conditions would be accompanied by increased MT disulfide bond formation. Given that there is virtually no free Zn in the cell [23], the released Zn from MT under oxidative conditions would transfer to other Zn-dependent proteins to regulate their functions. That MT bound Zn is transferred to Zn-binding proteins under oxidative conditions has been demonstrated in cell-free systems [23-28]. This transfer is possibly through a direct interaction between MT and Zn-binding peptide and protein [29,30], which provides a novel insight into the possible role of MT in cellular function.

MT as a potent antioxidant protecting cells from oxidative damage has been discussed and reviewed in previous papers [11-15,31-34]. As a non-specific free radical scavenger, MT has been proven more effective in quenching a wide range of free radicals including the most active radicals, hydroxyl radical and peroxynitrite than the specific ones [14,31-34]. This unique functional role of MT is derived from its unique structural features. The disulfide bond formation and the concomitant Zn release under conditions of oxidative stress contribute most likely to the cellular function of MT as a promising antioxidant in various diseases including diabetes.

MT PREVENTS DIABETES

Experimental Evidence

The first study to show the preventive effect of pancreatic MT synthesis on chemically-induced diabetes was carried out by Yang and Cherian [4]. In that study, the synthesis of pancreatic MT was induced by subcutaneous Zn injection in Sprague Dawley rats and then 12 hr later, diabetes was induced by a single injection of STZ. Results, summarized in (Fig. **2**), indicated that both Zn and MT levels increased in the rat pancreas in Zn alone and Zn + STZ groups on day 1 after STZ treatment. Serum MT also significantly increased in Zn + STZ group on day 1, 21 and 45 after STZ treatment. More importantly, serum glucose levels in both STZ and Zn + STZ group increased compared with the control group, but glucose levels in Zn + STZ group significantly lowered than that in the STZ group (Fig. **2D**). This result suggests that MT induction by Zn pre-treatment could significantly prevent STZ-induced diabetes. This important finding of MT prevention against diabetes onset was confirmed by several subsequent experiments, as summarized in Table **1**.

Zn was the most commonly used agent to induce pancreatic MT synthesis for the prevention of the onset of diabetes. Several approaches have been documented for Zn supplementation to induce pancreatic MT, which included subcutaneous or intraperitoneal injection, drinking water and dietary food. Cu is another well-known MT inducer [11,15]. Like Zn, supplementation of Cu also provided a significant prevention of diabetes induced by multiple low doses of STZ [35]. In addition, a transgenic mouse model in which MT is specifically overexpressed in the pancreas at about 20-fold higher than wild type (WT) was developed. These mice are highly resistant to STZ-induced diabetes [36], suggesting the direct protection of MT from diabetes.

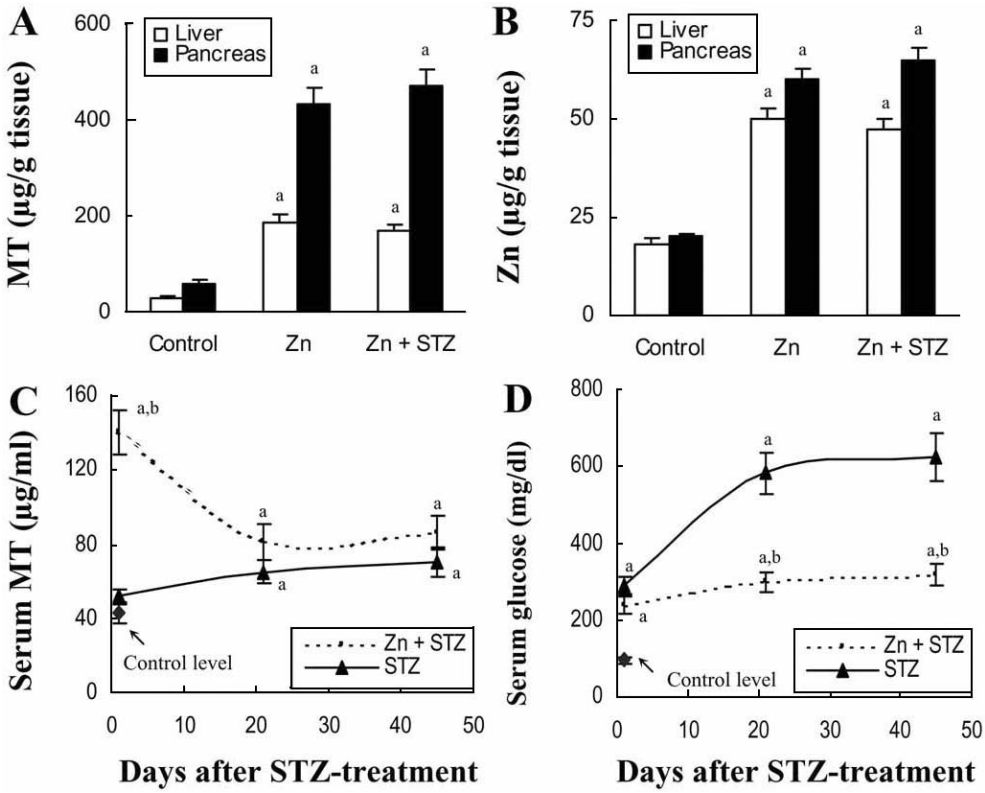

Fig. (2). Changes of Zn and MT contents in tissues, and MT contents and glucose levels in serum. Rats were treated by Zn (10 mg/kg) for 12 hr and then treated with STZ (65-80 mg/kg). On day 1 (30 hr) after STZ, MT (A) and Zn (B) contents were measured in pancreas and liver. In addition, serum MT (C) and glucose (D) levels were also measured on day 1, 21 and 45 after STZ treatment. a: $p<0.05$ *vs*. control; b: $p<0.05$ *vs*. STZ alone. This graph was modified based on previously published results [4] with permission from the authors.

MT induction was confirmed by measuring MT protein level using biochemical assay (such as Cd-hemoglobin saturation method), Western blotting or immunohistochemical localization and mRNA level using RT-PCR method. By *in situ* immunohistochemical methods, MT induction has been specifically identified in islet β-cells.

Induced MT synthesis prevents the onset of diabetes in a wide range of diabetic models. It was reported that multiple low doses of STZ induce a diabetic model mechanistically different from that induced by single high dose of STZ [35,37,38]. Induced MT synthesis in pancreas not only prevented diabetes induced by a single dose of STZ or alloxan, but also the one induced by multiple low doses of STZ. Moreover, MT induced by Zn was also found to protect diabetes development in genetically pro-diabetic models such as BB Wister rats and db/db or od/od mice [39-41]. This suggests that MT can prevent both type 1 and type 2 diabetes.

The preventive effect of MT on diabetes has been shown in different strains of rat or mouse models. Although there was a difference in the induction of diabetes by STZ between different strains of mice, protective effect of induced MT in pancreas was similar between strains. For example, C57BL/6 mice started severe hyperglycemia 2 weeks after the first injection of STZ, while B6SJL/F1 mice showed a long latent period for the development of a severe hyperglycemia about 10 weeks after the first injection of STZ [38]. However, MT induction in pancreas was protective in both diabetic models.

Clinical Evidence

Marchesini *et al.* [42] demonstrated the improvement of glucose disposal in cirrhotic patients either with diabetes or impaired glucose tolerance. They found that Zn levels reduced before treatment, but eventually normalized by oral Zn supplementation along with a significant improvement of glucose metabolism. Such finding was also demonstrated by a recent study conducted on type 2 diabetic patients [43], which was a placebo controlled and double-blind clinical trial with 46 type 2 diabetic patients. The patients were treated for 90 days with single daily doses of both Zn and melatonin; Zn and melatonin in combination with a regularly-used metformin, and placebo, respectively. The fasting plasma glucose and glycated hemoglobin were measured before starting the treatment (zero time) and after 30 and 90 days of treatment. Daily administration of Zn and melatonin significantly reduced fasting glucose and glycated hemoglobin levels, suggesting the importance of Zn supplementation in insulin signaling or glucose metabolisms.

However, not all studies supported this notion. Several studies indicated no improvement in the glucose metabolisms in either type 1 or type 2 diabetic patients after Zn treatment [44-46]. These contradictory results might have

Table 1. Evidence for the Preventive Effect of MT on Diabetes

Experimental Conditions	Major Mechanisms	Animals & Type of Diabetes	Outcomes	References
Zn pretreatment				
i.p. injection (10 mg/kg)	MT induction	Rats, STZ single dose (75 mg/kg)	++	[4]
Drinking (20 mM), 8 w	MT(ND)	ob/ob mice	++++	[39]
Dietary (1000 ppm), 4 w	MT(ND)	Pro-diabetic BB Wister rats	++++	[41]
Drinking (25 mM), 1 w	MT induction	C57BL/6 & B6SJL/F1 mice 5 x 40 mg STZ/kg	++++	[38]
Dietary (300 ppm), 6 w Dietary (1000 ppm), 2 w	MT(ND) MT induction	db/db mice CD-1 mice, ALX (50 mg/kg) STZ (5 x 40 mg/kg)	++++ ++++ ++++	[40] [37]
Drinking (25 mM), 1 (12) w	MT(ND)	C57BL/6, ALX (50 mg/kg)	+++	[75]
Drinking (25 mM), 1 w	Inhibiting NF-κB &/or AP1	NOD, C57BL/6 mice	++++	[76]
Cu pretreatment				
$CuSO_4$ (25 μg/0.2ml.mouse), 4 w	ND	C57BL/6, STZ (5 x 40 mg/kg)	++++	[35]
Transgenic mice				
Genetic enhancing MT	20-fold	MT-TG mice, STZ (1 x 200 mg/kg)	++++	[36]

Notes: ND, not detected; IS, immunohistochemical staining; WB, Western blotting; w, week; Cd-hem, [109] Cadmium-hemoglobin method to measure MT protein; 1 (12) w, 1 week prior to STZ and continued 12 weeks after STZ; ALX, alloxan.

emerged from a variety of factors, such as patient diversity and Zn speciation.

Possible Mechanisms

MT as an Antioxidant

Oxidative stress is the critical initiator of diabetes, and antioxidants can prevent its onset [2]. It is believed that overgeneration of ROS and RNS formation, resulting in damage to β-cells is a major causative factor in type 1 diabetes developed spontaneously through T cell-mediated inflammatory autoimmune reaction or induced by chemicals such as STZ and alloxan. The fact that low concentrations of antioxidants in animal pancreatic islets contribute to this organ's vulnerability to oxidative damage has been appreciated [47,48].

Pancreas contains relatively high levels of MT compared to other tissues such as liver and heart [49-51]. The high expression of MT in pancreas may suggest its requirement for normal physiological function. Indeed, mice with MT-I and -II-null are at a high risk of developing obesity and hyperlipidemia [52]. Furthermore, despite the similar insulin content, the islets from the MT-null mice showed much lower levels of basal and maximal insulin release than WT mice did [53]. These results suggest the requirement of MT for physiological function of pancreatic β-cells in which MT may act as an antioxidant in scavenging ROS and RNS to protect the protein sulfhydryls or nucleic acids, leading to preserving the cell membrane integrity and secretory responsiveness. Induction of MT by Zn in pancreatic β-cells has been shown to significantly protect against STZ-induced diabetes in rats and mice (Table **1**), suggesting that MT plays an important role in the prevention of oxidative injury that initiates diabetes. The possibility that the prevention of diabetes was due to the direct effect of inducers such as Zn can be excluded because pancreas-specific MT-overexpressing transgenic mice were resistant to spontaneously developed or STZ-induced hyperglycemia [36]. In cultured islets from control and pancreatic-specific MT transgenic mice, MT decreased STZ-induced islet disruption, DNA breakage and depletion of NAD^+. These results demonstrate that MT can reduce diabetes through inhibition of STZ-induced oxidative DNA damage [36].

Activation of NF-κB (a ROS-sensitive transcription factor) plays a critical role in initiating β-cell death leading to the onset of diabetes [37,54]. MT plays a role in negative regulation of NF-κB [55,56], whereas dietary Zn supplementation with the induction of pancreatic MT was found to significantly attenuate hyperglycemia in pro-diabetes db/db mice and alloxan- or STZ-induced diabetes, accompanied by significant inhibition of NF-κB activation [37,40].

In further support of the antioxidant action of MT, Kubisch *et al*. [57] showed that mice with upregulated SOD in the pancreas were highly resistant to chemically-induced diabetes. Thioredoxin (TRX), a redox (reduction/ oxidation)-active protein, has been shown to protect cells from oxidative stress. Transgenic mice with specific expression of TRX in pancreatic islets also showed a significantly lower incidence of spontaneously developed and STZ-induced diabetes as compared to WT counterparts [58]. In addition, low-dose radiation was found to upregulate antioxidant capacity leading to a wide range of adaptive response [59] and concomi-

tantly prevented against spontaneous or chemically-induced development of diabetes through enhanced pancreatic antioxidant capacity [60,61].

MT as a Regulator of Zn Homeostasis

With the exposure of the target tissues to oxidative stress, Zn bound to MT can be released and may then also play a protective role in the prevention of diabetes [62]. High Zn content was found in islets as compared to other tissues [63]. Several physiological roles of Zn in insulin function have been indicated [63-67]: (a) Zn is required for insulin forming hexameric crystals, which are stored in β-cells and released into the portal venous system at the time of β-cell degranulation; (b) Ratio of Zn to insulin within these crystals determines the ternary structure and antigenic properties of insulin and insulin binding capacity to its receptor; (c) Zn is closely involved in insulin-dependent metabolism of protein, carbohydrate and lipids, and the chelation of Zn is capable of inducing diabetes [66,68]; (d) Zn increases plasma insulin-like growth factor (IGF-1) contents [69]; and (e) Zn is an important factor in preventing cell death. Zn can inhibit caspase-3 activation to prevent cell death initiated by Fas/FasL pathway or mitochondrial cytochrome *c* pathway [70]. β-cell death induced by various insults plays a critical role in the development of diabetes. Therefore, it has been clearly highlighted that Zn plays important and multiple functions in regulating insulin function and preventing spontaneous and chemically-induced diabetes.

However, whether these functions of Zn are MT-dependent is unclear, although MT is normally found in high concentrations in pancreas and is also highly inducible by Zn. MT-null mice were used to investigate the effect of Zn supplement against STZ-induced diabetes in order to dissect the protective role against STZ-induced diabetes either by MT or Zn [71], but the results are still inconclusive. In that experiment, Zn pretreatment was given at two dose levels: i.e. 1 mg/kg and 10 mg/kg body weight as $ZnSO_4$. The high-dose of Zn fully suppressed the development of hyperglycemia in both MT-null and WT mice after STZ-treatment. However, the low-dose Zn-pretreatment had significantly inhibitory effect on STZ-induced hyperglycemia in MT-null mice, but no marked effect in WT control. This study seems to indicate that Zn itself plays an important role in the prevention of diabetic pathogenesis. In fact, the different effects between low-dose and high-dose levels of Zn may be due to the differing inducibility of MT in different tissues. It has been reported that supplementation to mice with Zn at 10 mg/kg induced comparable levels of MT between pancreas and liver of mice [72]. Therefore, it has been revealed that high dose of Zn can prevent STZ-induced diabetes in both MT-null and WT mice, since pancreas can gain certain doses of Zn except for liver in which Zn would be stocked due to its binding to MT. In contrast, low dose of Zn could not prevent STZ-induced diabetes in the WT mice probably because there was no free Zn available to pancreas after Zn was stocked in the liver where MT was induced by Zn, while low dose of Zn could prevent STZ-induced diabetes in MT-null mice since there was free Zn available for pancreas due to no hepatic MT induction. These results suggest that both Zn and MT are critical for the prevention of diabetes.

The fact that glucose-stimulated insulin secretion in MT-null mice is less than that in WT mice also suggests the requirement of pancreatic MT for the insulin secretion under normal condition [53]. In addition, the important role of MT in preventing diabetes was also supported by other indirect evidence. Enhanced expression of MT by inducers other than Zn such as cytokines [53] and Cu [35,73] also provides a significant prevention from diabetes, suggesting the antioxidant action of MT to play a significant role in the prevention of diabetes. Recently, there was a clinical observation [74] in which novel -209 A/G MT2A polymorphism was analyzed. Patients with -209 A/A MT2A genotype were shown to be at greater risk of developing diabetes along with a Zn deficiency than patients with -209 A/G MT2A genotype. Therefore, MT plays a critical role in coordination with Zn for the prevention of diabetes development, as outlined in (Fig. **3**).

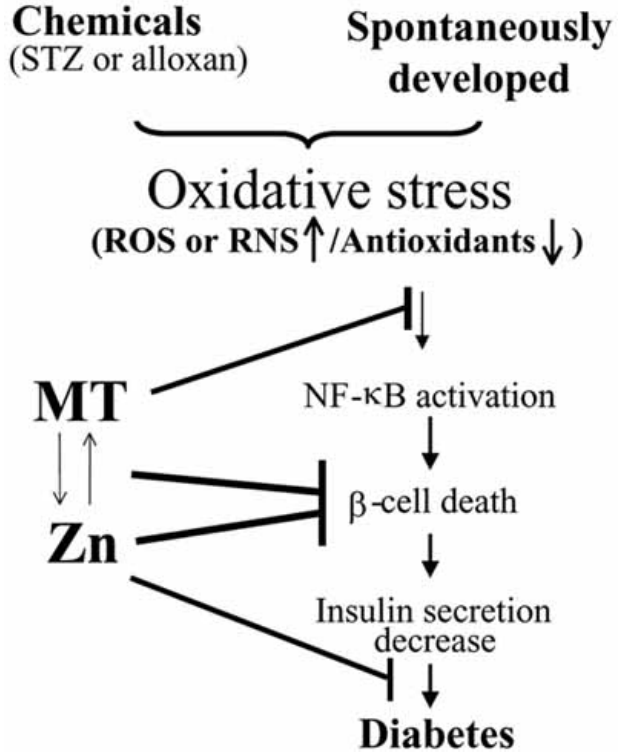

Fig. (3). Outline of mechanisms by which MT coordinates with Zn to prevent the onset of diabetes.

MT PREVENTS DIABETIC COMPLICATIONS

Chronic complications of diabetes, including several organs' injury and dysfunction, are the major lethal effects on diabetic patients. Chronic complications are associated with a variety of factors, including poorly controlled diabetes, hypertension, hyperlipidemia, inflammation and anemia, but the precise causative mechanism(s) remain unknown. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of diabetic complications [1,2]. Since MT is a potent, endogenous and inducible antioxidant in various tissues of human body, we have hypothesized that MT may act as an effective antioxidant in preventing diabetic complications, as indicated in (Fig. **4**).

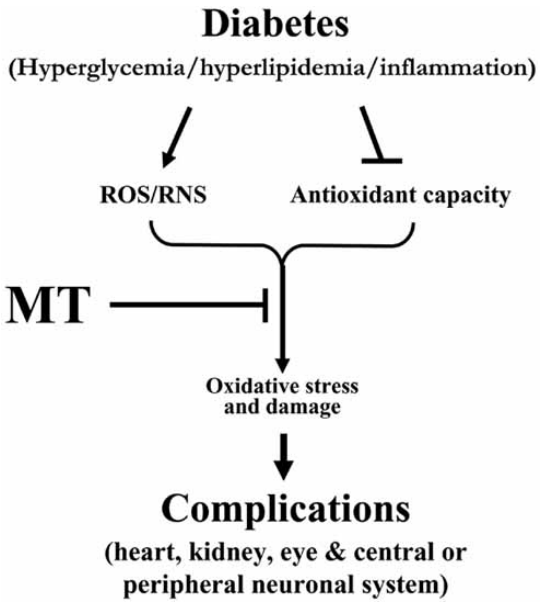

Fig. (4). Scheme of the mechanisms responsible for MT's prevention of diabetic complications.

Diabetic Cardiomyopathy

Preventive effect of MT on diabetes-induced cardiac toxicity was reported in our preliminary studies [5,6]. Diabetes-caused cardiac toxicities, measured by serum creatine phosphokinase (CPK) and cardiac cell death, were found to be significantly less in the heart of cardiac-specific MT-overexpressing transgenic mice. Exposure of cardiac cells to high levels of glucose induced significant ROS formation [77]. Therefore, diabetes-caused oxidative stress was proposed to play a critical role in inducing cell death in the diabetic hearts, leading to the late cardiac dysfunction, and it was also depicted that MT as a potent antioxidant might prevent diabetic cardiomyopathy through the suppression of diabetic oxidative damage. This notion was proved by the late studies from other groups (Dr. Epstein's lab [78,79] and Dr. Ren's lab [80-82]) as well as the author's own group [83-85]. We found that MT prevention of diabetic cardiomyopathy is mainly due to its suppression of diabetes-derived nitrosative stress and damage [86]. Table **2** summarizes these studies.

Diabetic Nephropathy

Diabetic nephropathy is another cause of mortality of diabetic patients and its prevention by MT has been demonstrated in animal model [88]. OVE26 mouse proved to be a transgenic model of severe early-onset type 1 diabetes, and was shown to develop hyperglycemia within the first week of life but survived well over a year with no insulin treatment with near normal body weight. By 2 months of age, OVE26 mice exhibited pronounced polyuria and significant albuminuria. Albumin excretion rate increased progressively with age and exceeded 15,000 μg/24 hr at 9 months of age. Structural studies showed an almost two-fold increase in kidney weight between 2 and 5 months along with progressively enlarged glomeruli, expanded mesangium with diffuse and nodular expansion of mesangial matrix and tubulointerstitial fibrosis [87]. If these genetic diabetes mice were crossbred with podocyte-specific MT-transgenic mice, the double-transgenic mice were produced and these mice have about 20-fold higher for MT specifically in the kidney and also spontaneously develop diabetes shortly after birth. The double transgenic mice showed a significant delay for diabetes induced through these renal pathogeneses [88]. This study provides the direct evidence that renal MT can prevent diabetes-related nephropathy.

Other Diabetic Complications

Protective effect of MT on diabetes-induced other complications has been investigated relatively less than that on diabetic heart and kidney.

Diabetes often causes bone calcium loss that is a major cause for diabetic fractures. Zn deficiency in the diet induces a reduction in the calcium content of diabetic bone of rats [89], but oral administration of Zn can significantly prevent

Table 2. MT Prevents Diabetic Toxicity in Cardiac Tissues

Experimental models	Major outcomes	References
Single STZ-induced diabetes in cardiac-specific MT-TG mice.	Increased serum CPK increase Increased cardiac oxidative damage Increased cardiac cell death Increased structural abnormalities in the heart Induce cardiac dysfunction in situ and cultured cardiomyocytes	[5,6,77] [81-83] [84]
OVE26MT mice *	Increased structural abnormalities in the heart Increased cardiac oxidative damage Induced cardiac dysfunction in Langendorff system	[78] [79]
High-fat food-induced insulin resistant (pre-diabetes) model in cardiac-specific MT-TG mice	Increased cardiomyocyte dysfunction in vitro model Increased cardiac oxidative damage Impaired insulin signaling	[80]

- The author previously produced a OVE26 diabetic mouse model (type-1 diabetes). They used this mouse model to crossbreed with a cardiac-specific MT-overexpressing transgenic mouse model. The OVE26MT mice are those in which cardiac MT is overexpressed about 20-fold and spontaneously develops diabetes [78].

the bone loss in STZ-induced diabetic animals [90,91]. Retinal MT gene depicted an increase when exposed to oxidative stress including diabetes and increased MT was found to act as a potent antioxidant against oxidative stress-induced retinal damage [92-94].

A recent study focusing on the biological response of brain to diabetes showed that brain MT was upregulated upon treatment with STZ, suggesting the possible protective role of MT in brain against oxidative stress [95]. In the brain, diabetes affects mostly the hippocampus and cerebellum as shown by a high GAFP immunopositivity of glial cells. Zn pre-treatment significantly reduced the observed astrocytosis. These results suggest the potential of Zn treatment for the induction of MT in the prevention of diabetic effects in the brain.

Clinical observations also provided the indirect evidence to support the possible protection of Zn-induced MT against diabetic complications. Two studies including either 50 cases [96] or 60 cases [97] of diabetic patients have indicated that oral Zn supplementation for 6 months significantly improved peripheral neuropathy as assessed by motor nerve conduction velocity. The use of Zn with melatonin together, or in combination with both melatonin and metformin, improves diabetes-induced microalbuminuria, an index of renal dysfunction, in type 2 diabetic patients [98]. Although tissue MT levels were not measured in these clinical studies, enhanced MT expression in the neuronal and renal tissues are expected and might be one of the mechanisms responsible for the neuronal and renal protection from diabetes.

In summary, all these preclinical or clinical studies support that Zn supplementation provides a protective mechanism against diabetes-induced cardiovascular complications, most likely through the induction of MT, as we proposed in (Fig. **4**).

CONCLUSIONS

Diabetes and its complications affect many populations worldwide. Understanding the mechanisms of development of diabetes, and developing the preventive approaches against its onset of diabetes and its complications are urgently needed. Emerging evidence has revealed that several mechanisms may be involved in the process, but oxidative stress as a unifying mechanism may be responsible for the development of both diabetes and its complications. Dietary Zn supplementation is effective for preventing or ameliorating diabetes in several rodent models of type 1 and type 2 diabetes. Mechanisms responsible for the prevention of type 1 diabetes may involve antioxidant mechanisms whether it is Zn alone (as an antioxidant), Zn-induced MT or both. Further studies are needed to identify the mechanism(s) for Zn protection from type 2 diabetes and determine whether Zn functions like insulin. Using transgenic mouse model, MT's direct protection against diabetes-induced cardiomyopathy and nephropathy was confirmed. However, whether the protection of Zn supplementation against other diabetic complications is also mediated by MT induction remains to be further investigated. Clinical contradictory outcomes are related to several influencing factors including patient status, supplemented Zn forms and MT expressions in diabetic individuals. Long-term clinical studies establishing safety and efficacy are required before recommendations can be made for patients presenting with diabetes.

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