Metallothionein deficiency exacerbates diabetic nephropathy in streptozotocin-induced diabetic mice

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Metallothionein deficiency exacerbates diabetic nephropathy in streptozotocin-induced diabetic mice. Am J Physiol Renal Physiol 306: F105–F115, 2014. First published October 23, 2013; doi:10.1152/ajprenal.00034.2013.—Oxidative stress and inflammation play important roles in diabetic complications, including diabetic nephropathy. Metallothionein (MT) is induced in proximal tubular epithelial cells as an antioxidant in the diabetic kidney; however, the role of MT in renal function remains unclear. We therefore investigated whether MT deficiency accelerates diabetic nephropathy through oxidative stress and inflammation. Diabetes was induced by streptozotocin injection in MT-deficient (MT−/−) and MT+/+ mice. Urinary albumin excretion, histological changes, markers for reactive oxygen species (ROS), and kidney inflammation were measured. Murine proximal tubular epithelial (mProx24) cells were used to further elucidate the role of MT under high-glucose conditions. Parameters of diabetic nephropathy and markers of ROS and inflammation were accelerated in diabetic MT−/− mice compared with diabetic MT+/+ mice, despite equivalent levels of hyperglycemia. MT deficiency accelerated interstitial fibrosis and macrophage infiltration into the interstitium in the diabetic kidney. Electron microscopy revealed abnormal mitochondrial morphology in proximal tubular epithelial cells in diabetic MT−/− mice. In vitro studies demonstrated that knockdown of MT by small interfering RNA enhanced mitochondrial ROS generation and inflammation-related gene expression in mProx24 cells cultured under high-glucose conditions. The results of this study suggest that MT may play a key role in protecting the kidney against high glucose-induced ROS and subsequent inflammation in diabetic nephropathy.

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DIABETIC NEPHROPATHY IS THE leading cause of end-stage renal disease worldwide and an independent risk factor for cardiovascular disease (10, 25). Several mechanisms contribute to the onset and progression of diabetic nephropathy, including genetic and hemodynamic factors, oxidative stress, and inflammation (4). Numerous studies have suggested that hyperglycemia is associated with enhanced generation of reactive oxygen species (ROS), and oxidative stress has been implicated in the development of diabetic nephropathy (6, 7). Emerging evidence also suggests that inflammatory pathways are crucially involved in the pathogenesis of diabetic nephropathy (20, 26). The regulation of oxidative stress and inflammation could thus represent a major therapeutic target in diabetic nephropathy. Metallothionein (MT) is an intracellular metal-binding protein characterized by a low molecular mass (6−7 kDa), high cysteine content (20 of 61–62 amino acids), and no aromatic or histidine residues (30). Although four isoforms have been characterized, MT-1 and -2 (MT-1/2) are widely distributed as the major isoforms throughout the body (30). MT plays an important role in heavy metal detoxification and essential metal homeostasis (24). In addition, MT has a potent antioxidant function and is an adaptive protein that protects cells and tissues from oxidative stress (2, 9). Previous studies have reported neuroprotective effects of MT in mouse models of Parkinson’s disease (5, 17, 18). We recently demonstrated that MT was expressed mainly in renal proximal tubular epithelial cells and that high-glucose-induced oxidative stress may enhance the expression of MT in the diabetic kidney (21). These results suggest that MT is upregulated in compensation to protect kidneys from oxidative stress induced by diabetic conditions; however, the role of MT in the pathogenesis of diabetic nephropathy remains poorly understood.

The present study therefore aimed to investigate the role of MT in protecting the kidney from high-glucose-induced oxidative stress under diabetic conditions, using MT deficient (MT−/−) and MT+/+ mice. We also used murine proximal tubular epithelial (mProx24) cells cultured under normal- or high-glucose conditions to determine whether knockdown of MT by small interfering RNA (siRNA) induced mitochondrial ROS, leading to inflammation.

MATERIALS AND METHODS

Experimental protocol. Male homozygous MT-1/2 knockout (MT−/−) mice were obtained from Jackson Laboratory (Bar Harbor, ME). The MT−/− mice were raised on a 129/Sv genetic background, and 129/Sv mice were therefore used as wild-type controls (MT+/+). All procedures were performed according to the Guidelines for Animal Experiments at Okayama University Medical School, the Japa-
nese Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Eight-week-old mice were divided into four groups: 1) non-diabetic MT+/+ mice (ND-WT; n = 7); 2) streptozotocin (STZ)-induced diabetic MT+/+ mice (DM-WT; n = 7); 3) non-diabetic MT−/− mice (ND-KO; n = 7); and 4) diabetic MT−/− mice (DM-KO; n = 7). Diabetes was induced and confirmed as reported previously (15). All mice had free access to a standard diet and tap water. Mice were euthanized at 12 wk after the induction of diabetes. The kidneys were removed, weighed, and fixed in 10% formalin for Metabolic data at 12 wk after induction of diabetes.

### Metabolic data at 12 wk after induction of diabetes

<table>
<thead>
<tr>
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<th>Nondiabetic</th>
<th>Diabetic</th>
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<tr>
<td></td>
<td>MT+/+</td>
<td>MT−/−</td>
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<tr>
<td></td>
<td>MT+/+</td>
<td>MT−/−</td>
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<tr>
<td>Urinary albumin excretion, μg/day</td>
<td>23.45 ± 2.43</td>
<td>19.01 ± 2.50</td>
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<tr>
<td>Body weight, g</td>
<td>30.45 ± 0.27</td>
<td>30.15 ± 0.57</td>
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<tr>
<td>Kidney weight, mg</td>
<td>430 ± 1.6</td>
<td>426 ± 1.4</td>
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<tr>
<td>Relative kidney weight, mg/g body weight</td>
<td>14.11 ± 0.41</td>
<td>14.12 ± 0.34</td>
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<tr>
<td>Glycated hemoglobin, %</td>
<td>3.60 ± 0.04</td>
<td>3.37 ± 0.06</td>
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Values are means ± SE; MT, metallothionein. *P < 0.01 vs. nondiabetic MT+/+; †P < 0.01 vs. diabetic MT+/−; ‡P < 0.05 vs. diabetic MT+/−.

**Immunoperoxidase staining.** Immunoperoxidase staining was performed as described previously (22). Briefly, fresh frozen sections were cut at 4-μm thickness using a cryostat. Macrophage infiltration was evaluated using a rat anti-mouse monoclonal/macrophage (F4/80) monoclonal antibody (Abcam, Cambridge, UK), followed by a biotin-labeled goat anti-rat IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). The avidin-biotin coupling reaction was performed on sections using a Vectastain Elite kit (Vector Laboratories, Burlingame, CA). We counted the number of F4/80-positive cells in 10 glomeruli/animal. The mean number of positive cells per glomerulus and interstitial tissue (number per mm²) were used for the estimation.

**Immunofluorescent staining.** Immunofluorescent staining was performed as described previously (21). Renal expression of MT-1/2 was detected using a rabbit anti-MT antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by Alexa Fluor 488 donkey anti-rabbit IgG (Invitrogen, Carlsbad, CA). To determine whether MT-1/2 was localized in proximal or distal tubular epithelial cells, the sections were counterstained with goat anti-aquaporin-1 (AQP1) antibody (Santa Cruz Biotechnology) or goat anti-Tamm-Horsfall protein (THP) antibody (Santa Cruz Biotechnology), respectively, followed by Alexa Fluor 594 donkey anti-goat IgG (Invitrogen). For other immunofluorescent staining, anti-type IV collagen (Millipore, Temecula, CA), anti-fibronectin (Sigma-Aldrich, St. Louis, MO), and anti-4-hydroxynonenal (4-HNE; Abcam) were used. Fluorescence images were obtained using a fluorescence microscope (BX51; Olympus, Tokyo, Japan).

**Western blot analysis.** Western blotting was performed as previously described (15). Briefly, the proteins were eluted, resolved by SDS-PAGE, and transferred to nitrocellulose membranes. After blocking in 20 mM Tris HCl (pH 7.6) containing 150 mM NaCl, 0.1% Tween 20, and 5% (wt/vol) nonfat dry milk, the membranes were incubated with anti-fibronectin (Sigma-Aldrich) and anti-phospho-NF-κB-p65 (Cell Signaling Technology, Danvers, MA). The membranes were hybridized with anti-β-actin (Abcam) to monitor equivalent loading in different lanes. All experiments were repeated at least three times.

**Electron microscopy.** Sections were prepared for electron microscopy as described previously (22). The proximal tubular epithelial cells were photographed at magnifications of ×1,500 and ×4,000.
Quantitative analysis of renal cortex gene expression. RNA was isolated from the renal cortex 12 wk after treatment using an RNeasy Mini kit (Qiagen, Valencia, CA). Single-strand cDNA was synthesized from the extracted RNA using an RT-PCR kit (PerkinElmer, Foster City, CA). Quantitative RT-PCR (qRT-PCR) was performed to analyze the mRNA expression of CD14, monocyte chemoattractant protein (MCP)-1, transforming growth factor (TGF)-β, and osteopontin (OPN) in the renal cortex, using StepOnePlus (Applied Biosys-
tems, Tokyo, Japan) and FastStart SYBR Premix Ex Taq II (Takara Bio, Otsu, Japan). The primers were purchased from Takara Bio. The results of qRT-PCR were evaluated by the comparative Ct method using GAPDH as the invariant control gene.

Mitochondrial ROS detection. Mitochondrial ROS were detected using MitoTracker Red CM-H2XRos (Molecular Probes, Eugene, OR) and MitoTracker Green FM (Molecular Probes). Briefly, renal sections were incubated with 10 μM MitoTracker Red CM-H2XRos and MitoTracker Green FM at room temperature for 1 h. Unbound dye was then removed, and fluorescence was analyzed using a fluorescence microscope (BX51; Olympus, Tokyo, Japan).

Cell culture and treatment. Murine proximal tubular epithelial (mProx24) cells were generously provided by Dr. Takeshi Sugaya (CMIC) and cultured as reported previously (21). mProx24 cells were cultured in DMEM supplemented with 5.5 mM d-glucose (low glucose), 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. siRNA experiments were performed using MT siRNA (sc-35926; Santa Cruz Biotechnology) and scrambled siRNA (sc-37007; Santa Cruz Biotechnology). mProx24 cells were transfected with 12.5 nM MT siRNA or scrambled siRNA in the presence of Lipofectamine RNAiMAX (Invitrogen). After siRNA transfection for 24 h, the cells were stimulated with 25 mM d-glucose (high glucose) for 24 h. Individual experiments were repeated at least three times with different lots or preparation of cells.

Quantitative analysis of gene expression in mProx24 cells. Total RNA was prepared from cells using an RNeasy Mini kit (Qiagen) as described above. Nox4, MCP-1, TGF-β, and OPN mRNA expression levels in mProx24 cells were measured using qRT-PCR, as described above.

ELISA. Levels of MT-1/-2 in kidney tissue and mProx24 cells were measured by an ELISA system (Frontier Science, Ishikari, Japan) according to the manufacturer’s protocol.

Statistical analysis. All values are given as means ± SE. Statistically significant differences between groups were examined using one-way ANOVA followed by Scheffé’s test. A P value <0.05 was considered statistically significant.

RESULTS

STZ induced greater renal damage in MT−/− mice compared with MT+/+ mice. The metabolic data are summarized in Table 1. The UAE was significantly higher in diabetic mice than in nondiabetic mice. Moreover, UAE was significantly increased in diabetic MT−/− mice compared with diabetic MT+/+ mice at 12 wk after STZ injection (120.66 ± 18.66 vs. 92.92 ± 14.76 μg/day; p < 0.05). Glycated hemoglobin and relative kidney weight were increased and body weight was decreased in diabetic mice compared with nondiabetic mice. There were no significant differences in glycated hemoglobin, relative kidney weight, and body weight between diabetic MT+/+ mice and diabetic MT−/− mice.

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**Fig. 2.** MT deficiency promotes macrophage infiltration into the interstitium in diabetic mice. A: macrophage infiltration into the glomeruli and the interstitium was clearly evident in diabetic mice and was significantly increased in DM-KO compared with DM-WT in the interstitium. Original magnifications: ×400 for glomeruli and ×100 for interstitium. B: number of intraglomerular macrophages. Values are means ± SE. **P < 0.01. C: number of macrophages in the interstitium. Values are means ± SE. *P < 0.05. **P < 0.01.
STZ induced more severe interstitial fibrosis in MT\(^{-/-}\) mice compared with MT\(^{+/+}\) mice. Kidneys were isolated and processed for pathological analysis using PAM staining, Masson’s trichrome staining, and immunofluorescence for type IV collagen and fibronectin (Fig. 1A). As revealed by PAM and type IV collagen staining, glomerular hypertrophy and mesangial matrix expansion were clearly observed in both MT\(^{+/+}\) and MT\(^{-/-}\) mice after STZ injection. However, morphometric analysis of PAM and the type IV collagen-positive area showed no significant differences between MT\(^{-/-}\) and MT\(^{+/+}\) mice (Fig. 1, B and C). Masson’s trichrome staining showed significantly increased interstitial fibrosis in diabetic MT\(^{+/+}\) mice, which was further increased in diabetic MT\(^{-/-}\) mice (1.58 ± 0.13 vs. 2.18 ± 0.16%; \(P < 0.05\)) (Fig. 1D). Immunofluorescent staining and Western blotting for fibronectin also showed the same tendency (Fig. 1, E and F). Furthermore, interstitial fibrosis in diabetic MT\(^{-/-}\) mice was attenuated by insulin treatment, suggesting that interstitial fibrosis observed in STZ-induced diabetic mice was mediated by hyperglycemia (Fig. 1, G and H). Collectively, these results demonstrate that MT deficiency accelerates interstitial fibrosis in STZ-induced diabetes.

Macrophage infiltration into the interstitium was increased in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice. The numbers of macrophages in both the glomeruli and interstitium were remarkably higher in diabetic mice compared with nondiabetic mice (Fig. 2A). There was no difference in macrophage infiltration into the glomeruli in response to STZ treatment between MT\(^{-/-}\) and MT\(^{+/+}\) mice (Fig. 2B). However, macrophage infiltration into the interstitium was increased in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice (2.50 ± 0.34 vs. 1.23 ± 0.18; \(P < 0.01\)) (Fig. 2C). These findings indicate that MT deficiency accelerates macrophage infiltration into the interstitium of the diabetic kidney.

MT-1/2 expression was increased in diabetic MT\(^{+/+}\) mice. We determined the levels of MT-1/2 in kidney tissue by ELISA (Fig. 3A). The MT-1/2 level in renal tissue was strongly upregulated in diabetic MT\(^{+/+}\) mice compared with control MT\(^{+/+}\) mice. In contrast, MT-1/2 was hardly detected in the kidneys of control and diabetic MT\(^{-/-}\) mice. To localize MT-1/2 in the diabetic MT\(^{+/+}\) kidney, we performed double immunofluorescence for MT-1/2, and AQP1 (a marker of proximal tubular epithelial cells) or THP (a marker of distal tubular epithelial cells). As shown in Fig. 3B, MT-1/2 was predominantly localized in the proximal tubular epithelial cells, and to a lesser extent in the distal tubular epithelial cells of the diabetic kidney.

Macrophage and inflammatory gene expression in the renal cortex were higher in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice. qRT-PCR analyses of kidney tissues demonstrated increased gene expression of the macrophage marker CD14 in the diabetic groups, and expression of this gene was increased in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice (Fig. 4A). Similarly, diabetes induction increased the renal expression of several proinflammatory and proatherogenic genes, including MCP-1, TGF-β, and OPN (Fig. 4, B–D). Notably, MCP-1 is a key chemokine involved in macrophage recruitment that plays a significant role in diabetic nephropathy, and the absence of MCP-1 significantly reduces diabetic renal injury. Similarly, TGF-β and OPN are also critical inflammatory cytokines involved in diabetic nephropathy. To investigate the involvement of NF-κB in the induction of inflammatory cytokines, we performed Western blotting. The expression of NF-κB was increased in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice (Fig. 4E). Collectively, these data indicate that MT deficiency accelerates diabetes-induced macrophage recruitment and inflammatory gene expression in the kidney.

Renal ROS generation was increased in diabetic MT\(^{-/-}\) mice compared with diabetic MT\(^{+/+}\) mice. To evaluate oxidative stress in the kidney, renal sections were immunostained for 4-HNE. This revealed that ROS were generated predominantly in tubular epithelial cells of diabetic MT\(^{+/+}\) and MT\(^{-/-}\) mice,
and to a lesser extent in the interstitium of nondiabetic MT+/+ and MT−/− mice (Fig. 5A). Moreover, we assessed mitochondrial ROS production using MitoTracker Red CM-H2XRos and MitoTracker Green FM staining. The intensity of MitoTracker Red CM-H2XRos was higher in diabetic MT+/+ mice compared with diabetic MT−/− mice (Fig. 5B). These findings suggest that MT deficiency increases diabetes-induced mitochondrial ROS in the interstitium of the kidney.

Mitochondrial morphology deteriorated in diabetic MT−/− mice. To confirm the beneficial effects of MT on generating mitochondrial ROS, we examined renal morphology in more detail using electron microscopy. The number of swollen mitochondria was increased, and cristae were also prominently reduced in renal proximal tubular cells of diabetic MT+/+ mice compared with nondiabetic MT+/+ mice, and were further increased in diabetic MT−/− mice compared with diabetic MT+/+ mice (Fig. 6). These results indicate that MT deficiency impaired mitochondrial function in renal proximal tubular epithelial cells in the diabetic kidney.

ROS and inflammatory gene expression levels were increased by knockdown of MT in cultured proximal tubular epithelial cells. Mouse mProx24 renal proximal tubular epithelial cells were transfected with MT siRNA or scrambled siRNA as a control and subjected to qRT-PCR analyses and ELISA. MT mRNA and protein expression was significantly inhibited in MT knockdown cells compared with control cells (Fig. 7A and B). High-glucose-induced Nox4 mRNA expression was increased in MT knockdown cells (Fig. 7C). To evaluate mitochondrial ROS in the mProx24 cells, we performed double staining using MitoTracker Red CM-H2XRos and MitoTracker Green FM. The intensity of MitoTracker Red CM-H2XRos was increased in mProx24 cells transfected with MT siRNA (Fig. 7G). Similarly, expression levels of inflammatory genes including MCP-1, TGF-β, and OPN were upregulated by MT RNAi (Fig. 7D–F).
7, D–F). These findings suggest that knockdown of MT exacerbates high-glucose-induced oxidative stress and inflammation in renal proximal tubular epithelial cells.

**DISCUSSION**

In the present study, we demonstrated that MT deficiency accelerated albuminuria and interstitial fibrosis without affecting blood glucose levels in STZ-induced diabetic mice. Macrophage infiltration in the interstitium of the diabetic kidney and the expression of inflammatory genes, including MCP-1, TGF-β, and OPN, were increased in diabetic MT/−/− mice. Furthermore, mitochondrial ROS were increased and mitochondria were fragmented in diabetic MT/−/− mice. In vitro studies with proximal tubular epithelial cells revealed that knockdown of MT increased the expression of Nox4, which was associated with oxidative stress, and expression of inflammatory genes. Our findings suggest that MT has antioxidative and anti-inflammatory effects in diabetic kidneys and prevents the development of diabetic nephropathy, independently of blood glucose levels.

MT comprise a family of low-molecular-weight, cysteine-rich, ubiquitous, and inducible intracellular proteins that bind to heavy metals such as zinc, copper, and cadmium and participate in metal homeostasis and detoxification (1). The mammalian MT family comprises four isoforms: MT-1, MT-2, MT-3, and MT-4. MT-3 is predominantly brain specific and is expressed in neurons and stimulated glial cells (16), while the two major isoforms, MT-1/-2, are expressed in most organs. However, the expression of MT in the kidney has been unclear. This and our recent study demonstrated that MT-1/-2 were highly induced in renal proximal tubular epithelial cells in diabetic mice (Fig. 3) and rats (21). This study also showed that MT has previously been reported to be a potent antioxidant; protecting cells from oxidative damage (2, 5, 9, 17, 18). We therefore hypothesized that MT may be induced in the kidney as an antioxidative protein and thus protect the kidney from diabetes-induced ROS and inflammation.

Many studies have proposed an important role of oxidative stress in the pathogenesis of diabetic nephropathy (3, 11, 29). We evaluated oxidative stress in the kidney by assessing mitochondrial ROS generation. MitoTracker Red CM-H2XRos and MitoTracker Green FM staining revealed that mitochondrial ROS were increased in the interstitium, mainly in the tubular epithelial cells, of diabetic MT/−/− mice compared with...
diabetic MT<sup>+/+</sup> mice. Electron microscopy also showed more severe mitochondrial swelling in proximal tubular epithelial cells in diabetic MT<sup>−/−</sup> mice compared with diabetic MT<sup>+/+</sup> mice. It has been reported that mitochondrial morphology is altered in renal diseases including diabetic nephropathy (8, 31, 32). Since MT is a potent antioxidant and adaptive protein that protects cells and tissues from oxidative stress (12), we speculated that MT deficiency is likely to contribute to increased mitochondrial swelling in diabetic MT<sup>−/−</sup> mice. However, no studies have reported how MT is related to mitochondrial morphology, and thus further studies are needed. We also performed siRNA experiments to explore the effects of MT on the gene expression level of Nox4, as a promoter of ROS generation, and on the mitochondrial ROS in cultured proximal tubular epithelial cells. The fact that Nox4 expression and the intensity of MitoTracker Red CM-H2XRos were increased by knockdown of MT suggests that MT may prevent Nox4-derived ROS generation by reducing oxidative stress in proximal tubular epithelial cells. Overall, these results indicate that MT deficiency increases diabetes-induced oxidative stress in the interstitium of the kidney.

Inflammation is also associated with the development of diabetic nephropathy (13, 15, 19). The present study revealed that increased expression levels of the macrophage marker CD14, the chemokine MCP-1, and the cytokines TGF-β and OPN noted in diabetic MT<sup>+/+</sup> mice were further increased in diabetic MT<sup>−/−</sup> mice. Similarly, macrophage infiltration into the interstitium and interstitial fibrosis were increased in diabetic MT<sup>−/−</sup> mice compared with diabetic MT<sup>+/+</sup> mice. However, macrophage infiltration in the glomeruli and mesangial matrix accumulation were similar in both types of mice. Moreover, in vitro studies demonstrated that the expression levels of inflammatory genes including MCP-1, TGF-β, and OPN were increased by knockdown of MT in cultured proxi-
mal tubular epithelial cells. These results indicate that MT deficiency involves inflammation in the interstitium, but not in the glomeruli, in diabetic nephropathy. The expression of NF-κB, a master regulator of inflammatory genes, is increased in diabetic MT−/− mice compared with diabetic MT+/+ mice. This result suggests that MT may suppress high-glucose-induced inflammation by inhibiting NF-κB.

We and others (14, 27) have demonstrated that MT plays a protective role in chronic kidney injury models induced by cadmium or cisplatin in the MT knockout mouse, but no studies have been reported in experimental models of diabetic nephropathy in the MT knockout mouse. Although we have previously shown that MT is upregulated in the kidney of the STZ-induced diabetic rat (21), whether MT prevents diabetes-
induced oxidative stress and inflammation, and thus protects against diabetic nephropathy, remains unclear. Podocyte-specific MT-transgenic mice showed that overexpression of MT in podocytes could ameliorate the primary features of diabetic nephropathy (33), suggesting that protection of the podocytes could inhibit diabetic nephropathy. Induction of renal tubular MT synthesis by zinc supplementation also prevents diabetic nephropathy by acting against oxidative stress (23, 28). Our current and previous study (21) showed that MT is induced mainly in renal tubules, rather than podocytes, in the diabetic kidney. Therefore, MT in renal proximal tubular epithelial cells might thus be a therapeutic target for the treatment of diabetic nephropathy.

In conclusion, we demonstrated that MT deficiency accelerates high-glucose-induced oxidative stress and inflammation in the kidney. The results of this study indicate that MT plays an important role in protecting the kidney from diabetic stress by acting as an antioxidant protein. Our findings suggest that MT might be a novel therapeutic target for the treatment of diabetic nephropathy.

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DISCLOSURES

D. Ogawa and A. Nakatsuka belong to the Department of Diabetic Nephropathy, endowed by Astellas and Boehringer Ingeheim. J. W. is a consultant for Boehringer Ingeheim and receives speaker honoraria from Novartis. H. M. is a consultant for AbbVie and Astellas, receives speaker honoraria from Astellas, MSD, Takeda, and Tanabe Mitsubishi, and receives grant support from Astellas, Daiichi Sanyko, Dainippon Sumitomo, MSD, Novo Nordisk, and Takeda. The authors report no conflicts of interest in this work.

AUTHOR CONTRIBUTIONS


REFERENCES


