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H_2S improves renal fibrosis in STZ-induced diabetic rats by ameliorating TGF-β1 expression

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ABSTRACT

Nephropathy develops in many patients with type 1 diabetes mellitus (T1DM). However, the specific mechanisms and therapies remain unclear. For this purpose we investigated the effects of hydrogen sulfide (H_2S) on renal fibrosis in streptozotocin (STZ) induced diabetic rats and its underlying mechanisms.

Experimental rats were randomly divided into four groups: Control group (normal rats), DM group (diabetes rats), DM + NaHS group [diabetes rats treated with sodium hydrosulfide (NaHS)], and NaHS group (normal rats treated with NaHS). The diabetic models were established by intraperitoneal injection of STZ. The NaHS-treated rats were injected with NaHS as an exogenous donor of H_2S. At the same time, control group and DM group were administrated with equal doses of normal saline (NS). After eight weeks, the rats' urine samples were collected to measure the renal hydroxyproline content by basic hydrolysis method with a hydroxyproline detection kit. Collagen I and III content was detected by immunohistochemical method, and the pathology morphology of kidney was analyzed by Masson staining. Protein expressions of transforming growth factor beta 1 (TGF-β1), ERK1/2, TIMP1, TIMP2, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 were assessed by western blotting. The results showed that significant fibrosis occurred in the kidney of diabetes rats. NaHS treatment downregulated TGF-β1, ERK1/2, TIMP1, TIMP2, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 expressions in the kidney of these diabetes rats ($p<.01$). This result suggests that NaHS treatment could attenuate renal fibrosis by TGF-β1 signaling, and its mechanisms may be correlated with ERK1/2 expression and modulation of MMPs/TIMPs expression. Therefore, H_2S may provide a promising option for defensing against diabetic renal fibrosis through TGF-β1 signaling, equilibrating the balance between profibrotic and antifibrotic mediators.

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Hydrogen sulfide; diabetic renal fibrosis; transforming growth factor beta 1; extracellular signal-regulated kinase-1/2; matrix metalloproteinases/tissue inhibitors of metalloproteinases

Introduction

Diabetic nephropathy (DN), which may be the cause of disability, is one of the severe microvascular complications of diabetes. Therefore, how to prevent the progression of DN has become a hot focus and challenge.

According to domestic and overseas researches, renal fibrosis is one of the final major pathways of DN that may cause renal failure and subsequently lead to sudden death.[1] And the main morphological changes, associated with the loss of kidney function in diabetic kidney, include overproduction, deposition and contraction of extracellular matrix (ECM) in the glomeruli.[1] Collagen and fibronectin are the primary matrix proteins observed.[2] In diabetic kidney, all of these proteins are significantly increasing in mesangial cells (MCs), resulting in glomerulosclerosis.[3]

In DN, the increased expression of transforming growth factor beta 1 (TGF-β1) has been shown to promote accumulation of ECMs such as collagens and fibronectin,[4] apoptosis,[5] dedifferentiation of podocytes,[6] and epithelial–mesenchymal transition of proximal tubules,[7] all of which are considered to facilitate renal hypertrophy and dyfunction.[8] Extracellular signal-regulated kinase-1/2 (ERK1/2), a member of the mitogen-activated protein kinase (MAPK) family, may express in MCs in the condition of high glucose.[9] And, researches have demonstrated that ERK1/2 may upregulate TGF-β1 expression.[10] Also, previous studies have proven that dysregulation of matrix metalloproteinases (MMPs)/tissue inhibitors of metalloproteinases (TIMPs) is involved in the process of renal fibrosis.[11] MMPs, an endogenous family of enzymes, are responsible for

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ECM degradation. MMPs and their physiological inhibitors TIMPs construct a time-and-space dependent system. It may play a crucial role during the production of renal fibrosis and its progression to remodeling.

In this study, we aimed to explore the role of TGF-β1 and the possible regulating mechanism among TGF-β1, ERK1/2, and MMPs/TIMPs *in vivo*.

Materials and methods

Diabetes model induction

Adult male SD rats (280 ± 40 g), were purchased from the SJA Lab Animal Center of Changsha (Changsha, PR China). All animals were bred in sub-cages in a clean grade laboratory environment in a constant temperature condition (23 ± 1 °C), and accepted 12 h artificial lighting for acclimatization. All rats freely consumed water and food. After one week, the 52 experimental rats were divided into four groups ($n = 13$): control group (normal rats), DM group (diabetes rats), DM + NaHS group (diabetes rats treated with NaHS), and NaHS group (normal rats treated with NaHS). Diabetes was induced by a single intraperitoneal injection of 40 mg/kg STZ (MP Biomedicals LLC, Santa Ana, CA), which was dissolved quickly in 0.1 mol/L sodium citrate buffer 72 h after STZ injection.

Only those rats with a blood glucose level ≥16.7 mmol/L were considered as successful diabetic models. The rats accepting the injection were administered 5% glucose water within 24 h to prevent hypoglycemia shock.

A total of 12 rats were sacrificed before and after modeling, and concentrated in the two weeks after modeling. The overall survival rate of rats was 77% (40/52). In the control group, DM group, DM + NaHS group, and NaHS group, the numbers of successful survival models were 12, 9, 9, and 10, respectively. Rats' mental state in the control group was good and responsive, maintaining white and shiny coats. While diabetic rats showed polydipsia, polyphagia, polyuria, and weight loss and some other symptoms like unresponsiveness and dull fur. After the modeling, DM + NaHS group and NaHS group accepted intraperitoneal injection of NaHS solution at a dose of 100 μmol/kg daily, while the control group and DM group were injected intraperitoneally the same dose of NS daily for eight weeks. This study was approved by the Animal Care Committee of the University of South China and conformed to the Guide for the Care and Use of Laboratory Animals.

Blood sample and tissue collection

After eight weeks, all rats were sacrificed by intraperitoneal injection of chloral hydrate (350 mg/kg). Then the rats' thoracic cavities were opened and were perfused intracardially with NS and fixative 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The kidney of each rat was removed and fixed in the same fixative for 12 h and then placed in 30% phosphate-buffered sucrose until the tissue sank.

Histopathological examination and immunohistochemical assay

Kidneys were fixed in 4% paraformaldehyde and were embedded in paraffin, sectioned and stained with Masson staining, and at last observed at ×400 magnification under optical microscope. The expressions of collagen I and III were determined by immunohistochemistry. Slides were deparaffinized in xylene and hydrated in ethanol gradient. The tissue sections, treated with 3% hydrogen peroxide (H_2O_2) solution for 15 min, were washed with phosphate buffered saline (PBS) three times at room temperature. The antigen was recovered by boiling for 15 min in tris-ethylenediaminetetraacetic acid buffer in microwave oven at high power. After cooling, the slides were washed for three times in PBS. Then, primary antibody was incubated for 1 h at 37 °C. These sections were washed and incubated with horseradish peroxidase (HRP)-labeled goat anti-rabbit/mouse polyclonal antibodies at 37 °C for 10 min, and 3,3 diaminobenzidine (DAB) was used for color reaction. The reaction was washed with distilled water twice before stopped, and then stained with hematoxylin.

Specimen collection and processing

At the end of the eight weeks, 24 h urinary protein (Ualb) and 24 h urinary microalbumin excretion were determined by biochemical methods.

Hydroxyproline content assay

Kidney hydroxyproline content was measured by basic hydrolysis method with a hydroxyproline detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing Shi, PR China). The 10 mg pieces of tissue was mixed with HCl and added to 1 ml basic hydrolysates in oven at 120 °C overnight. Then, the hydrolysates were neutralized and mixed with chloramine T solution and oxidized for 20 min at room temperature. The oxidized product reacted with p-dimethylaminobenzaldehyde in

ethanol and H_2SO_4 solution at 60 °C for about 25 min. And the resulting chromophore was quantified spectrophotometrically at 557 nm against a standard curve of known hydroxyproline concentration.

Western blot analysis

A frozen kidney tissue of rats in each group was taken to extract proteins and estimate the protein by bicinchoninic acid assay (BCA) colorimetric method. The protein was denatured and separated by SDS-PAGE electrophoretic technique and transferred to a polyvinylidene difluoride (PVDF) membrane. After formulating with 5% bovine serum albumin (BSA) for 2 h, the blocked membranes were incubated with ERK1/2 (cell signaling), TGF-β1, MMP-2, MMP-7, MMP-8, MMP-11, MMP-14, TIMP1, and TIMP2 (Boster Biological Technology, Ltd., Wuhan, PR China) antibody, and then washed with tris buffered saline with Tween (TBST), and detected with a secondary antibodies (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), and finally these membranes were subjected to chemiluminescence detection assay.

Statistical analysis

SPSS version 18.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. Values are presented as mean ± SD. Statistical differences between the groups were assessed by one-way ANOVA with SPSS version 18.0 software. Difference was considered to be statistically significant if $p<.05$.

Results

Masson staining of kidney tissues

See Figure 1(A) for the normal tissue structures and thickness of the basement membrane in kidneys of the normal group. The part made by dyeing blue showed collagen fiber. We observed that the accumulation of collagen fiber was significantly massive in DM group. And the diabetic renal injury is characterized by mesangial expansion, global sclerosis, interstitial fibrosis, and tubular atrophy (Figure 1(B)). In the diabetic rats treated with NaHS, the findings of the injury was markedly reduced (Figure 1(C)). The normal rats that accepted NaHS injection presented the same architecture as the normal group (Figure 1(D)).

Immunohistochemistry study of collagen I and III in the kidney

Figure 2 shows collagen I (up) and III (down) expression in glomerulus of normal rats (Figure 2(A)) by immunohistochemistry study. The part made by dyeing brown showed collagen fiber. Compared with normal rats, the kidney of DM rats presented significantly higher levels of collagen I and III (Figure 2(B)).

However, the levels of collagen I and III were significantly decreased in DM + NaHS group (Figure 2(C)). The normal rats accepted NaHS injection presented the same as the normal group (Figure 2(D)).

Effects of H_2S on 24 h urinary protein and 24 h urinary microalbumin excretion (means ± SD) (Table 1)

At the end of the eight weeks, 24 h Ualb and 24 h urinary microalbumin excretion were detected. As shown in Table 1, compared with the normal group 24 h Ualb and 24 h urinary microalbumin excretion in the DM group were significantly increased ($p<.01$). Compared with DM group, 24 h Ualb and 24 h urinary

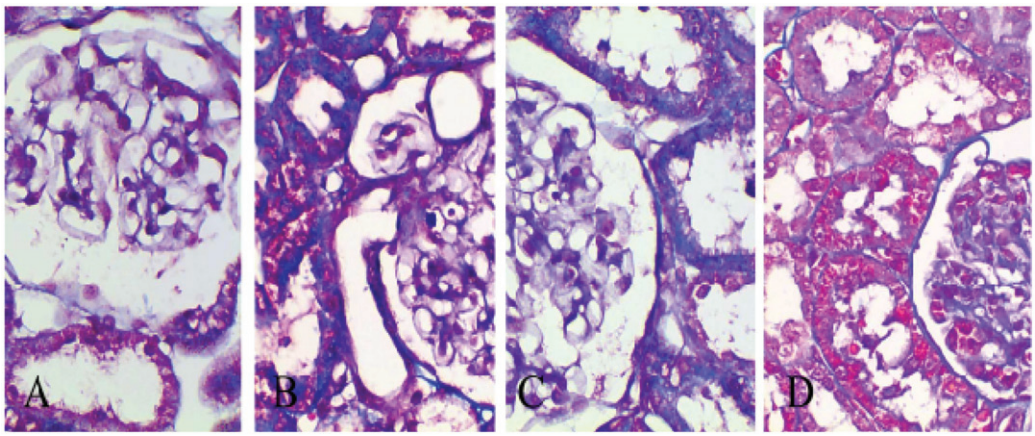

Figure 1. Masson staining of kidney tissues. (A) A rat in control group, (B) a rat in DM group, (C) a rat in DM + NaHS group, and (D) a rat in NaHS group.

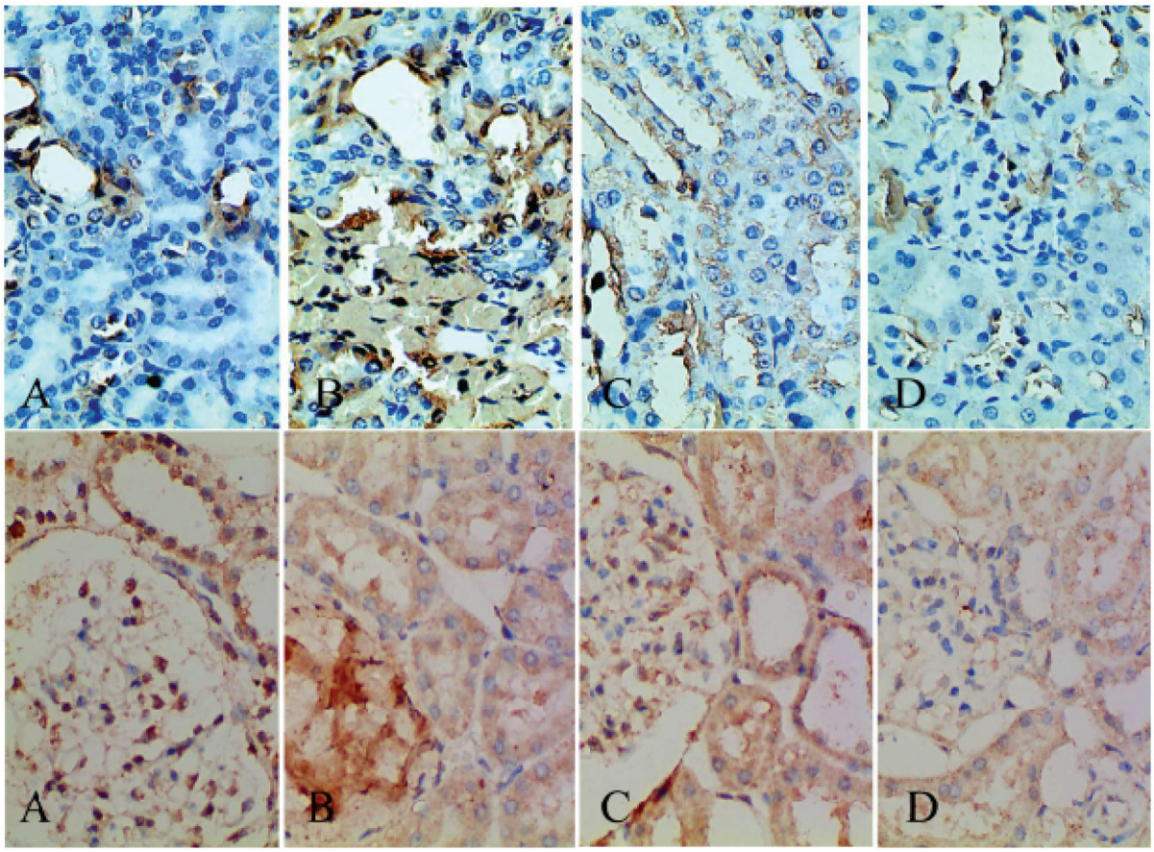

Figure 2. Immunohistochemistry study of collagen I (up) and collagen III (down) expression in kidney. (A) A rat in control group, (B) a rat in DM group, (C) a rat in DM + NaHS group, and (D) a rat in NaHS group.

Table 1. Effects of hydrogen sulfide on 24-h urinary protein and 24-h urinary microalbumin excretion (mean ± SD).

Groups	24-h urinary protein excretion	24-h urinary microalbumin excretion
Control group	11.8 ± 3.3	0.5 ± 0.3
DM group	44.5 ± 6.4[a]	13.0 ± 2.6[a]
DM + NaHS group	26.6 ± 6.1[b]	5.9 ± 2.4[b]
NaHS group	12.6 ± 4.0	0.6 ± 0.3

[a]$p<.01$ vs. control group.
[b]$p<.01$ vs. DM group.

microalbumin excretion in the DM rats treated with NaHS showed a marked decrease ($p<.01$), but it was still higher than the control group.

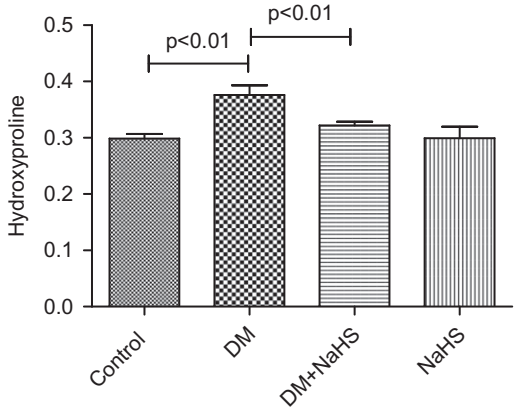

Figure 3. Hydroxyproline content in kidney of each group.

Effects of H_2S on hydroxyproline content in diabetes rats

Hydroxyproline content in kidney of each group are showed in Figure 3. Compared with control group, there was a significantly higher level of hydroxyproline in DM group ($p<.01$). However, compared with DM group, it decreased remarkably in DM + NaHS group ($p<.01$).

Effects of H_2S on TGF-β1 and ERK1/2 expressions in diabetes rats

As showed in Figure 4 TGF-β1 and ERK1/2 expressions in DM group were much higher than those in the control group ($p<.01$). However, compared with DM group,

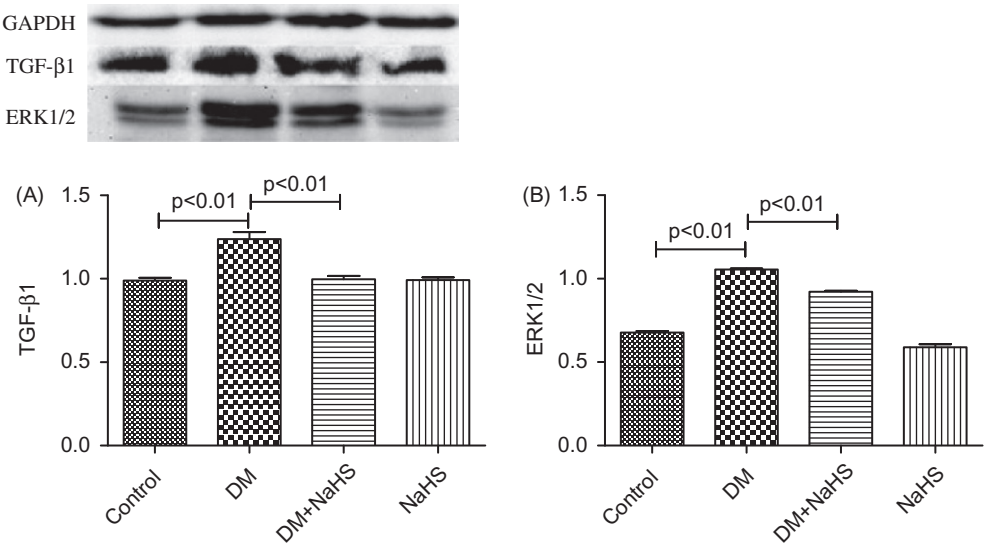

Figure 4. TGF-β1 (A) and ERK1/2 (B) expression in the renal tissues from each group (normalized by GAPDH).

TGF-β1 and ERK1/2 expressions were markedly decreased in DM + NaHS group ($p < .01$).

TIMP1 and TIMP2 expressions in each group

As showed in Figure 5, TIMP1 ($p < .05$) and TIMP2 ($p < .01$) expressions in DM group were much higher than those in the control group. While, compared with DM group, TIMP1 and TIMP2 expressions were significantly decreased in DM + NaHS group ($p < .01$), and TIMP1 expression was decreased in DM + NaHS group ($p > .05$).

MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 expression in each group

As showed in Figure 6, there was MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 expression were much higher than those in DM group ($p < .01$).

While, compared with DM group, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 expression were significantly decreased in DM + NaHS group ($p < .01$).

Discussion

Renal fibrosis is a common consequence of chronic renal disease such as DN, which may finally give rise to severe clinical complications and dysfunction. The histological features of renal fibrosis are excessive deposition of ECM and interstitial fibroblast. While, the typical pathological change of DN is glomerular sclerosis followed by thickening of renal tubule and glomerulus basement membrane, mesangial ECM expansion, hypernephrotrophy and renal tubule interstitial fibrosis.[11,12] While, MMPs, inhibited by tissue inhibitors of MMPs (TIMPs), are responsible for the degradation of ECM, and their substrates contain collagens, proteoglycans and many glycoproteins. MMP-7, a member of the MMP family, can cleave a series of substrates including collagen, fibronectin, and is largely regulated by TIMP2.[13] MMP-8 is deemed as neutral neutrophil collagenase mainly secreted by neutrophils. In condition of inflammation and wound healing process, the common expression of MMP-8 was significantly increased. Activated MMP-8 can decrease collagen matrix and damage its structure, giving rise to the expression of fibrosis and high expression of collagen I and III.[14,15] MMP-14 is a transmembrane protein, and MMP-11 can be soluble in cellular microenvironment. MMP-11 is a substrate of MMP-14, and it is able to prevent MMP-11 enzymatic activity. By hydrolyzing MMP-11, MMP-14 can provide space for restrictions and protease activity of MMP-11, and protections for specific and important cells surrounding ECM from MMP-11 in an inappropriate correlation. MMP-14, a membrane-bound metalloproteinase, is processed before its insertion into specific plasma membrane domains.[16] The major functions of MMP-14 include the degradation of some certain ECM components and the activation of proMMP-2,[17] which then decreases collagen IV, the primary component of the basement membrane.[18] TIMP2 combines either the active or latent form of MMP-2 with less inhibitory activity to other MMPs.[19] In our study, we found an obvious increase of dark stained collagenous fiber elements of ECM in renal interstitium of diabetic rats and an increase of collagen II in renal glomerulus and tubule interstitial. And there was a higher level of hydroxyproline in DN tissues. Meanwhile, the expressions of TIMP1,

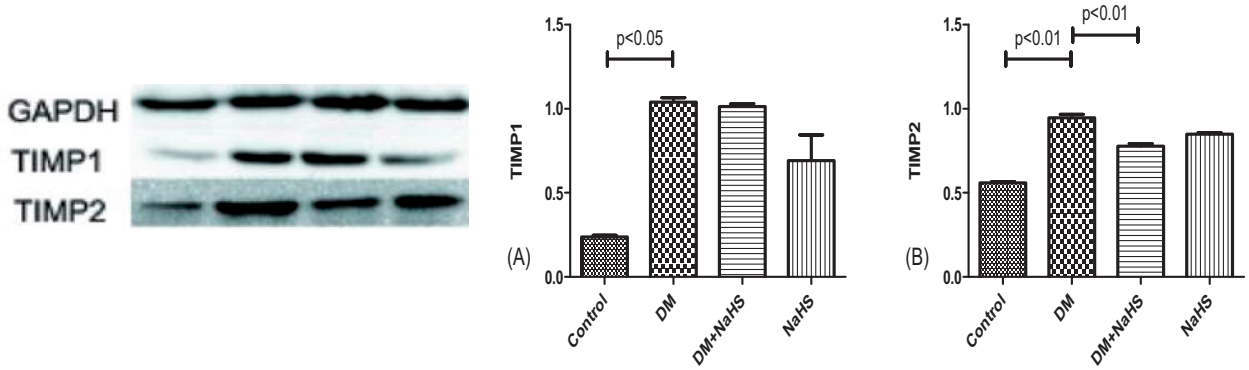

Figure 5. TIMP1 and TIMP2 expressions in the renal tissues from each group [normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH)].

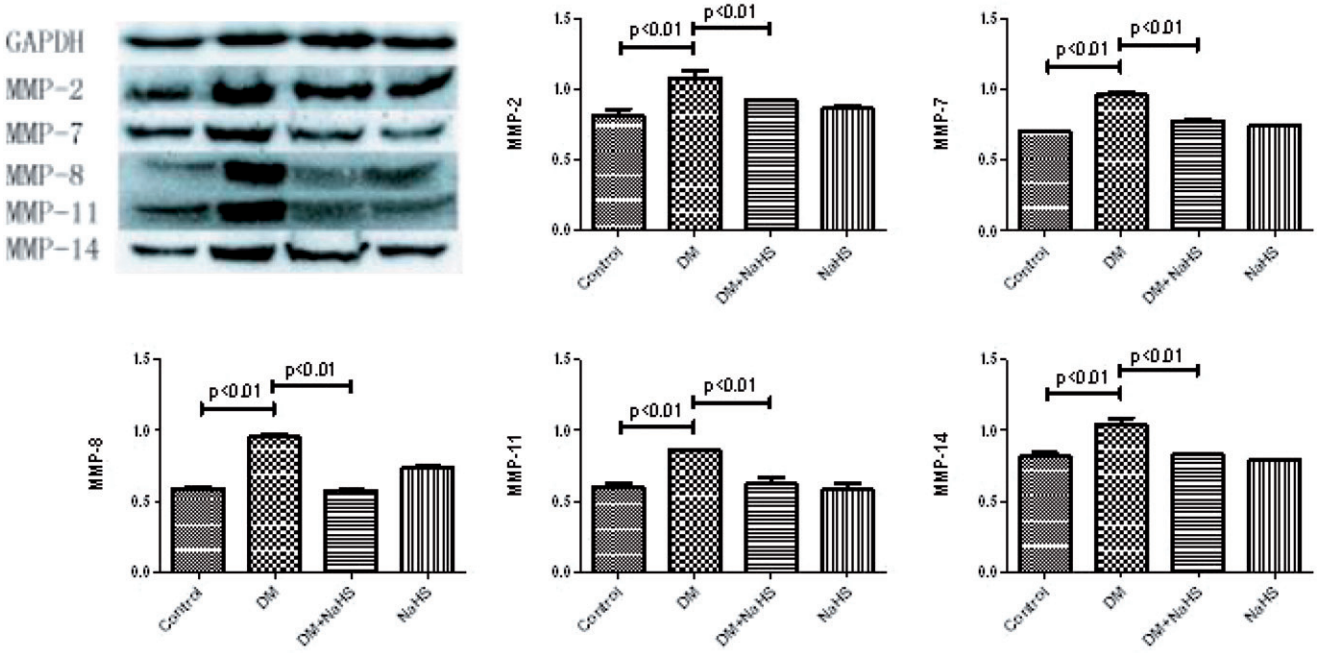

Figure 6. Expression of MMP-2, MMP-7, MMP-8, MMP-11, MMP-14 in the renal tissues from each group (normalized by GAPDH).

TIMP2, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14, which were publicly involved in the progress of renal fibrosis, were significantly increasing in diabetic rats. All these findings suggest apparent fibrosis of renal interstitium in diabetic rats and its relevance with the reduced degradation of metalloproteinases.

TGF-β family members play a crucial role in homeostasis of adult tissues.

A number of evidences demonstrate that lack of coordination of TGF-β-dependent signalings may result in some diseases such as fibrosis.[20] TGF-β1, as an important member of TGF-β family, is an acknowledged mediator in the triggering and progression of fibrosis and has been regarded as the key of ECM expression.[21,22] TGF-β1 has been described to be correlated with regulation of the expression and activity of some MMPs/TIMPs in different ways.[23] However, downstream TGF-β1 signaling occurs through canonical and non-canonical pathways. ERK1/2, which may be activated in MCs exposed to high glucose, acts as the downstream of TGF-β1 through the non-canonical pathways.[24] According to researches based on diabetic animal models, disruption of TGF-β1 signaling significantly attenuated glomerular mesangial matrix expansion.[25] TGF-β1 intervention can increase the degree of phosphorylated forms of ERK1/2.[26] Studies show that, by inhibiting TGF-β1 protein expression, NaHS failed to decrease the expression of fibronectin compared to the

group treated with NaHS without the inhibitors.[27] Also, previous studies have demonstrated that MMP-2 can be up-regulated by some stimuli such as TGF-β1, which in turn can activate the fibroblast-to-myofibroblast transition.[28] In this study, we verified augmented expression of TGF-β1 and ERK1/2 in the diabetes rats.

Therefore, we demonstrated that TGF-β1 may have a natural link with the pathogenesis of DN by inducing interstitial fibrosis via ERK1/2 pathway. H_2S, a kind of new gaseous signal, has been suggested to play a variety of necessary physiological and physiopathological roles.[29] It is generated from L-cysteine by several enzymes including cystathionine γ lyase (CSE) and cystathionine β synthase (CBS). Some studies have shown that H_2S biosynthesis is impaired in diabetes, and that it may be effective to administer different H_2S donors to diabetic animals.[30]

In this research, contrasted with DM group, we noted that the expression of collagen fibers in basement membrane and collagen II was distinctly decreased in DM + NaHS group. And 24 h Ualb and 24 h urinary microalbumin excretion were both decreased in DM + NaHS group. Meanwhile, the renal hydroxyproline of DM + NaHS group was markedly decreased. The expression levels of proteins, such as TGF-β1, ERK1/2, TIMP1, TIMP2, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 in renal tissues, were up-regulated significantly in the DM group, while the expressions of TGF-β1 and ERK1/2 protein were down-regulated in the DM + NaHS group, and the expressions of TIMP1, TIMP2, MMP-2, MMP-7, MMP-8, MMP-11, and MMP-14 also degraded notably. These all suggested that the mechanism by which H_2S improves renal tissue fibrosis. In summary, this study demonstrated that treatment with H_2S could attenuate the progression of renal dysfunction in diabetic rats. The protective effect of H_2S may be correlated with TGF-β1 signaling through ERK1/2 pathway. However, this proposed mechanism and its clinical prospect in DN protection still need further investigation in the future.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References

1. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. *J Clin Invest*. 1984;74:1143–1155.
2. Mason RM, Wahab NA. Extracellular matrix metabolism in diabetic nephropathy. *J Am Soc Nephrol*. 2003;14:1358–1373.
3. Lin CL, Wang JY, Ko JY, Huang YT, Kuo YH, Wang FS. Dickkopf-1 promotes hyperglycemia-induced accumulation of mesangial matrix and renal dysfunction. *J Am Soc Nephrol*. 2010;21:124–135.
4. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA*. 1993;90:1814–1818.
5. Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes*. 2006;55:225–233.
6. Herman-Edelstein M, Thomas SC, Thallas-Bonke V, Saleem M, Cooper ME, Kantharidis P. Dedifferentiation of immortalized human podocytes in response to transforming growth factor-β: A model for diabetic podocytopathy. *Diabetes*. 2011;60:1779–1788.
7. Zeisberg M, Hanai J, Sugimoto H, et al. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med*. 2003;9:964–968.
8. The Chronic Complications Investigation Group of the Diabetes Society of Chinese Medical Association. "The retrospective analysis about diabetes chronic complications and related macroangiopathy of nationwide in patient's from 1991 to 2000 in China". *J Chin Acad Med Sci Univ*. 2002;5:447–451.
9. Haneda M, Araki S, Togawa M, Sugimoto T, Isono M, Kikkawa R. Mitogen-activated protein kinase cascade is activated in glomeruli of diabetic rats and glomerular mesangial cells cultured under high glucose conditions. *Diabetes*. 1997;46:847–853.
10. Hayashida T, Decaestecker M, Schnaper HW. Crosstalk between ERK MAP kinase and Smad signaling pathways enhances TGF-beta-dependent responses in human mesangial cells. *FASEB J*. 2003;17:1576–1578.
11. Berthier CC, Wahl PR, Le Hir M, et al. Sirolimus ameliorates the enhanced expression of metalloproteinases in a rat model of autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant*. 2008;23:880–889.
12. Bai Y, Wang L, Li Y, et al. High ambient glucose levels modulates the production of MMP-9 and alpha5(IV) collagen by cultured podocytes. *Cell Physiol Biochem*. 2006;17:57–68.
13. Piotrowski WJ, Górski P, Pietras T, Fendler W, Szemraj J. The selected genetic polymorphisms of metalloproteinases MMP2, 7, 9 and MMP inhibitor TIMP2 in sarcoidosis. *Med Sci Monit*. 2011;17:598–607.
14. Ye S. Putative targeting of matrix metalloproteinase-8 in atherosclerosis. *Pharmacol Ther*. 2015;147:111–122.
15. Pei XM, Yung BY, Yip SP, et al. Protective effects of desacyl ghrelin on diabetic cardiomyopathy. *Acta Diabetol*. 2015;52:293–306.

16. Buache E, Thai R, Wendling C, et al. Functional relationship between matrix metalloproteinase-11 and matrix metalloproteinase-14. *Cancer Med*. 2014;3:1197–1210.
17. Wang H, Li Q, Shao L, et al. Expression of matrix metalloproteinase-2, -9, -14, and tissue inhibitors of metalloproteinase-1, -2, -3 in the endometrium and placenta of rhesus monkey (Macaca mulatta) during early pregnancy. *Biol Reprod*. 2001;65:31–40.
18. Rozanov DV, Savinov AY, Williams R, et al. Molecular signature of MT1-MMP: Transactivation of the downstream universal gene network in cancer. *Cancer Res*. 2008;68:4086–4096.
19. De Clerck Y, Yean T, Ratzkin B, Lu H, Langley K. Purification and characterization of two related and distinct metalloproteinase inhibitors secreted by bovine aortic endothelial cells. *J Biol Chem*. 1989;264: 17445–17453.
20. Hsieh H-L, Wang H-H, Wu W-B, Chu P-J, Yang C-M. Transforming growth factor-b1 induces matrix metalloproteinase-9 and cell migration in astrocytes: Roles of ROS-dependent ERK- and JNK-NF-κB pathways. *J Neuroinflamm*. 2010;7:88. doi: 10.1186/1742-2094-7-88.
21. Bottinger EP, Bitzer M. TGF-β signaling in renal disease. *J Am Soc Nephrol*. 2002;13:2600–2610.
22. Hewitson TD. Renal tubulointerstitial fibrosis: Common but never simple. *Am J Physiol Renal Physiol*. 2009;296: 1239–1244.
23. Gomes LR, Terra LF, Wailemann RA, Labriola L, Sogayar MC. TGF-β1 modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. *BMC Cancer*. 2012;12:26. doi: 10.1186/1471-2407-12-26.
24. Hayakawa Y, Aoyama T, Yokoyama C, et al. High salt intake damages the heart through activation of cardiac (pro) renin receptors even at an early stage of hypertension. *PLoS One*. 2015;10:e0120453. doi: 10.1371/journal.pone.0120453.
25. Wang A, Ziyadeh FN, Lee EY, Pyagay PE, Sung SH, Sheardown SA. Interference with TGF beta signaling by Smad3-knockout in mice limits diabetic glomerulosclerosis without affecting albuminuria. *Am J Physio Renal Physiol*. 2007;293:1657–1665.
26. Vivar R, Humeres C, Ayala CP, et al. TGF-β1 prevents simulated ischemia/reperfusion-induced cardiac fibroblast apoptosis by activation of both canonical and non-canonical signaling pathways. *Biochim Biophys Acta*. 2013;1832:754–762.
27. Guo L, Peng W, Tao J, et al. Hydrogen sulfide inhibits transforming growth factor–β1-induced EMT via Wnt-Catenin pathway. *PLoS One*. 2016;13:e0147018. doi: 10.1371/journal.pone.0147018.
28. Sun L, Jin H, Sun L, et al. Hydrogen sulfide alleviates myocardial collagen remodeling in association with inhibition of TGF-β/Smad signaling pathway in spontaneously hypertensive rats. *Mol Med*. 2015;20:503–515.
29. Chen R, Xue J, Xie M. Osthole regulates TGF-β1 and MMP-2/9 expressions via activation of PPARα/γ in cultured mouse cardiac fibroblasts stimulated with Angiotensin II. *J Pharm Pharm Sci*. 2013;16:732–741.
30. Wallace JL. Physiological and pathophysiological roles of hydrogen sulfide in the gastrointestinal tract. *Antioxid Redox Signal*. 2010;12:1125–1133.