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Hydrogen sulfide inhibits the renal fibrosis of obstructive nephropathy

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Hydrogen sulfide has recently been found decreased in chronic kidney disease. Here we determined the effect and underlying mechanisms of hydrogen sulfide on a rat model of unilateral ureteral obstruction. Compared with normal rats, obstructive injury decreased the plasma hydrogen sulfide level. Cystathionine-β-synthase, a hydrogen sulfide-producing enzyme, was dramatically reduced in the ureteral obstructed kidney, but another enzyme cystathionine-γ-lyase was increased. A hydrogen sulfide donor (sodium hydrogen sulfide) inhibited renal fibrosis by attenuating the production of collagen, extracellular matrix, and the expression of α-smooth muscle actin. Meanwhile, the infiltration of macrophages and the expression of inflammatory cytokines including interleukin-1β, tumor necrosis factor-α, and monocyte chemoattractant protein-1 in the kidney were also decreased. In cultured kidney fibroblasts, a hydrogen sulfide donor inhibited the cell proliferation by reducing DNA synthesis and downregulating the expressions of proliferation-related proteins including proliferating cell nuclear antigen and c-Myc. Further, the hydrogen sulfide donor blocked the differentiation of quiescent renal fibroblasts to myofibroblasts by inhibiting the transforming growth factor-β1-Smad and mitogen-activated protein kinase signaling pathways. Thus, low doses of hydrogen sulfide or its releasing compounds may have therapeutic potentials in treating chronic kidney disease.

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Tubulointerstitial fibrosis is the final common pathway to chronic kidney diseases (CKD).[1] Pathologically, renal fibrosis manifests as the formation of myofibloblasts and the deposition of extracellular matrix proteins in the renal interstitium.[2] The mechanisms of renal fibrosis have not been fully elucidated and effective drugs are still scarce. Renin–angiotensin–aldosterone system is one of the main culprits of renal fibrogenesis, and renin–angiotensin–aldosterone system inhibitors remain the first-line drugs in fighting renal fibrosis.[3] However, the renin–angiotensin–aldosterone system inhibitors may deteriorate renal function and cause hyperpotassemia when serum creatinine rises above 3 mg/dl.[4] New antifibrotic agents are therefore needed to expand therapeutic options and decrease side effects, which is especially important for azotemia patients.

Hydrogen sulfide (H_2S) represents the third gasotransmitter along with nitric oxide and carbon monoxide.[5] It is generated by cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulphurtransferase. CBS and CSE are enriched in renal proximal tubules and produce H_2S in kidney in a combined way.[6] H_2S plays various physiological and pathological roles in the kidney. For instance, it exhibits diuretic, natriuretic, and kaliuretic properties by increasing glomerular filtration rate and functions as an oxygen sensor in the renal medulla.[6,7] Recently, it was reported that plasma H_2S level was decreased in 5/6 nephrectomy rat and uremia patients,[8,9] suggesting that uremic toxin of CKD impairs the production of endogenous H_2S.

Notably, the biological functions of H_2S in CKD are not fully understood. Heterozygous *cbs*$^{-/-}$ mice with unilateral nephretomy, an animal model of CKD, developed proteinuria and collagen deposition, and increased the expressions of matrix metalloproteinase-2 and -9.[10] Emerging evidence also demonstrates that H_2S exhibits antifibrotic effects in the lung, heart, and liver.[11–13] Furthermore, H_2S bears some similarities to the other two gaseous molecules, nitric oxide and carbon monoxide, both of which protect the kidney from renal fibrosis.[14,15] Taken together, we hypothesize that H_2S may attenuate renal fibrosis.

In this study, we examined the effect of H_2S on unilateral ureteral obstructive (UUO) animal model and defined its safe and effective dosage range. Furthermore, we investigated the roles of H_2S in renal fibroblast proliferation and differentiation. The potential mechanisms were also explored.

RESULTS

CBS expression and plasma H_2S levels are decreased in the obstructed kidney

To investigate whether endogenous H_2S was involved in the pathogenesis of renal fibrosis, we examined the expression and activity of H_2S-producing enzymes CBS and CSE, as well as plasma H_2S levels. The CBS expression was nearly completely ablated by obstructive injury at day 14, whereas CSE was increased. In contrast, the expressions of CBS and CSE in the contralateral kidneys were not affected (Figure 1a–c). Moreover, H_2S generation in the obstructed kidneys was dramatically reduced compared with sham-operated rats (Figure 1d). Plasma H_2S levels were reduced by ~30% in UUO rats compared with the sham counterparts (27.5 ± 4.3 μmol/l vs. 39.4 ± 6.3 μmol/l, $P = 0.021$, Figure 1e). Immunohistochemistry staining indicated that CBS was predominantly expressed in proximal renal tubules. UUO injury time-dependently reduced CBS expression in the obstructed kidneys without affecting that in the contralateral and sham-operated kidneys (Figure 2a and b). In contrast, CSE was mainly located in renal glomeruli, interstitium, and interlobular arteries of normal rats. UUO injury markedly increased the CSE expression in the interstitium of obstructed kidneys. The CSE expression in the unobstructed kidneys remained unchanged (Figure 2c and d).

NaHS treatment decreases the renal size, increases the cortical thickness, and ameliorates renal function of UUO rats

To assess the effect of exogenous H_2S supplement on renal fibrosis, we treated UUO rats with incremental doses of sodium hydrosulfide (NaHS; 5.6, 56, and 560 μg/kg/day) and CSE inhibitor (DL-propargylglycine (PAG), 25 mg/kg/day) via intraperitoneal injection once daily for 3 days before and 2 weeks after UUO injury. The renin–angiotensin–aldosterone system inhibitor enalapril (10 mg/kg/day) was also administrated via intragastria as a positive control. Gross appearance (Figure 2a) and group data (Figure 2b and c) showed that enalapril and NaHS (56 μg/kg/day) reduced the size of the kidney and increased the renal cortical thickness in UUO rats. However, these effects were not observed in the rats treated with NaHS at a higher dose of 560 μg/kg/day or PAG (Figure 3). Compared with UUO rats, NaHS (56 μg/kg/day) decreased serum creatinine levels, whereas PAG increased serum urea nitrogen concentration. There were no differences of serum electrolytes among each group (Supplementary Data and Supplementary Table S1 online).

NaHS attenuates renal tubulointerstitial injury and collagen deposition in renal interstitium

Hematoxylin and eosin staining results indicated that UUO rats exhibited dilated renal tubule, epithelial cells atrophy, interstitial expansion, and increased infiltration of inflammatory

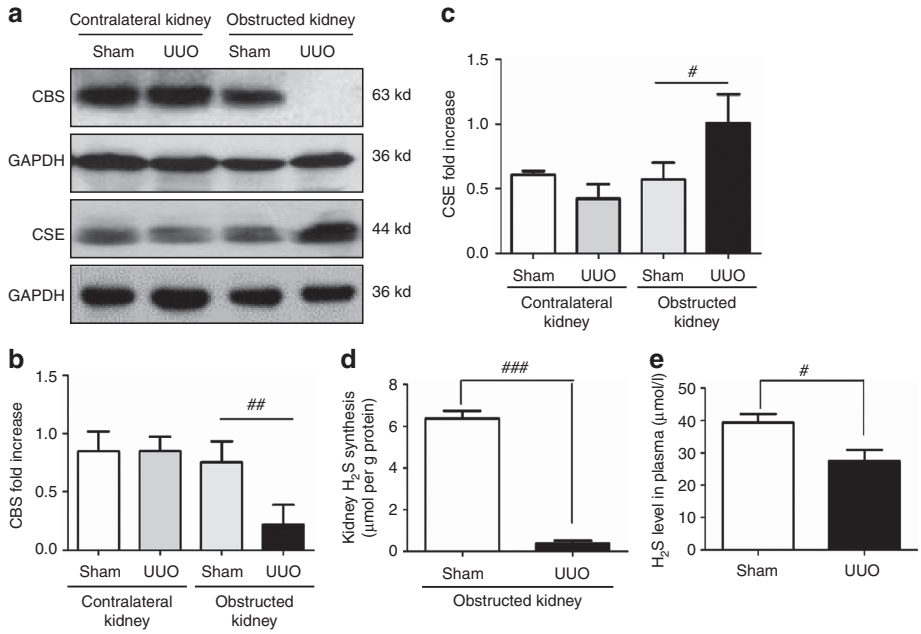

Figure 1 | Unilateral ureteral obstructive (UUO) injury downregulates cystathionine-β-synthase (CBS) expression and decreases hydrogen sulfide (H_2S) level in plasma on day 14 after operation. (**a**) Representative western blots of CBS and cystathionine-γ-lyase (CSE) proteins in sham and UUO group are shown. Relative levels of (**b**) CBS and (**c**) CSE were analyzed by normalizing to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (**d**) Kidney H_2S production and (**e**) plasma H_2S level in sham and UUO group are shown. Data are mean ± s.e.m., $n = 4$–6, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus sham group.