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HYDROGEN SULFIDE METABOLISM AND ITS ROLE IN KIDNEY FUNCTION IN A RAT MODEL OF CHRONIC KIDNEY DISEASE

METABOLIZM SIARKOWODORU ORAZ JEGO ROLA W FUNKCJONOWANIU NEREK W MODELU CHRONICZNEJ CHOROBY NEREK U SZCZURÓW

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Summary

Background. Chronic kidney disease (CKD) is an ongoing global problem. It is correlated with a substantial increase in mortality and morbidity. Although, hydrogen sulfide (H_2S) plays an important role in the physiological and pathological processes in the kidney, the influence of CKD on the enzymatic synthesis and utilization of H_2S in the kidney is unclear. The aim of this study was to evaluate the activity of H_2S-producing enzymes (cystathionine gamma-lyase, cystathionine beta-synthase and cysteine aminotransferase) and the content of H_2S in rats with CKD, and to establish the relationship between these parameters and markers of the functional state of the kidneys. **Material and methods.** CKD in rats was induced by 5/6 nephrectomy of the contralateral kidney. H_2S regulation was examined in post-nuclear kidney homogenates by measuring H_2S levels and cystathionine gamma-lyase, cystathionine beta-synthase, and cysteine aminotransferase activity using spectrophotometric methods. Functional and biochemical measurements were monitored after water load (5% of body mass). **Results.** CKD in rats was associated with defects in H_2S metabolism in rat kidneys. The activity of H_2S-producing enzymes decreased by 28.3-34.2% ($p<0.05$), the rate of utilization of exogenous H_2S in the kidneys increased by 34.3% ($p<0.05$), and the content of H_2S was reduced by 35.8% ($p<0.05$) in comparison with a control group. A progressive loss of kidney function (tubular and glomerular disorders) is closely correlated with the content of H_2S in the kidneys. **Conclusions.** The H_2S system in kidneys may be an important metabolic target, which can influence the efficacy of treatments to improve the functional state of kidneys in CKD.

Keywords: chronic kidney disease, hydrogen sulfide, cystathionine gamma-lyase, cystathionine beta-synthase, cysteine aminotransferase

Streszczenie

Wprowadzenie. Przewlekła choroba nerek (ang. chronic kidney disease, CKD) jest obecnie problemem na skalę globalną. Fakt ten jest skorelowany ze znacznym wzrostem zachorowalności i śmiertelności. Pomimo, iż siarkowodór (H_2S) odgrywa ważną rolę w procesach fizjologicznych i patologicznych zachodzących w nerkach, oddziaływanie przewlekłej choroby nerek na enzymatyczną syntezę i wykorzystanie H_2S w nerkach pozostaje niejasne. Celem niniejszego badania jest ocena aktywności enzymów wytwarzających H_2S (gamma-liazy cystationinowej, beta-syntazy cystationinowej i aminotransferazy cysteinowej), a także zawartości siarkowodoru u szczurów z przewlekłą chorobą nerek i ustalenie związku tych parametrów z markerami stanu funkcjonalnego nerek. **Materiał i metody.** Przewlekłą chorobę nerek u szczurów wywołano przez wykonanie niepełnej nefrektomii przeciwległej nerki. Regulacja siarkowodoru została oszacowana w homogenacie pojądrowym nerki przez pomiar poziomu H_2S, gamma-liazy cystationinowej, beta-syntazy cystationinowej oraz aminotransferazy cysteinowej za pomocą metod spektrofotometrycznych. Pomiary funkcjonalne i biochemiczne monitorowano po obciążeniu wodą (5% masy ciała). **Wyniki.** Przewlekła choroba nerek u szczurów wiąże się z zaburzeniami metabolizmu siarkowodoru w ich nerkach. Aktywność enzymów wytwarzających H_2S zmniejszyła się o 28,3-34,2% ($p<0,05$), szybkość wykorzystania egzogenicznego H_2S w nerkach wzrosła o 34,3% ($p<0,05$), a zawartość H_2S została zmniejszona o 35,8% ($p<0,05$) w porównaniu z grupą kontrolną. Postępująca utrata czynności nerek (zaburzenia kanalikowe i kłębuszkowe) jest ściśle skorelowana z zawartością H_2S w nerkach szczurów. **Wnioski.** Układ siarkowodoru w nerkach może stanowić ważny cel metaboliczny, który może wpływać na skuteczną terapię poprawiającą czynności nerek przy przewlekłej chorobie tego narządu.

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Słowa kluczowe: przewlekła choroba nerek, siarkowodór, gamma-liaza cystationinowa, beta-syntaza cystationinowa, aminotransferaza cysteinowa

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Introduction

Chronic kidney disease (CKD) is the loss of kidney function over the time (eGFR<60 mL/min/1.73 m^2 for three months or more). CKD is correlated with a substantial increase in mortality and morbidity worldwide [1-3]. World Health Organization experts called kidney diseases the most important noninfectious diseases of our time [4]. In 1990, CKD was ranked 27th among all causes of mortality; by 2010, its prevalence grew by approximately 82%, ranking 18th, which was the third-highest mortality rate among the top 25 causes of death (after HIV/AIDS – 39.6% and diabetes – 93%). It is worth noting that there was an increase in its prevalence and incidence all over the world [5]. According to the annual report by the USRDS (United States Renal Data System), stages 1-4 CKD is found in 30 million Americans, or 14.8% of the population [6]. According to data from the national register of patients with CKD, 465,641 patients with stages 1-4 CKD were registered in Ukraine as of January 1st, 2014 [7]. The molecular mechanisms of kidney damage are the subject of extensive research efforts. It is currently understood that several processes are involved and have a direct effect on certain cellular targets, subcellular structures, enzymes or transport proteins, oxidative and nitrosative stress, apoptosis, and inflammation [8-11]. However, the detailed mechanisms remain unclear. Therefore, the establishment of molecular mechanisms of the protective potential of excretory organs becomes of particular importance considering the possibility of identifying additional markers of nephrotoxicity and development of pathogenically valid approaches to the prevention and medical treatment of kidney damage.

Hydrogen sulfide (H_2S) is an important biologically active compound synthesized by the kidneys in scarce amounts, which regulates the basic physiological functions of the kidneys [12-15]. The production of H_2S in the kidneys is regulated by three main pyridoxal phosphate-dependent enzymatic systems: 1) cystathionine gamma-lyase (CSE) catalyzes H_2S production through hydrolysis of cysteine; 2) cystathionine beta-synthase (CBS) forms H_2S through cysteine and homocysteine condensation reactions; 3) cysteine aminotransferase (CAT) is involved in the production of H_2S through the transamination of cysteine by α-ketoglutarate. H_2S utilization in the kidneys occurs predominantly through oxidation reactions with sulfates and during the production of sulfates, which are eliminated with urine. H_2S plays several important biological functions in the kidneys: 1) acts as an antioxidant and cytoprotector; 2) stimulates filtration processes in the kidneys; 3) activates sodium and potassium excretion in urine; 4) reduces the activity of the renin-angiotensin-aldosterone system. Further, dysregulation of H_2S metabolism is the basis of kidney damage. However, there are contradictory data regarding the direction of H_2S metabolism changes in different kidney pathologies [16,17]. At present, there is virtually no data showing the effects of experimental chronic renal failure on the enzymatic synthesis and utilization of H_2S in the kidneys.

The purpose of the study was to evaluate the activity of H_2S-producing enzymes (CSE, CBS, and CAT), as well as the content of hydrogen sulfide in rats with chronic kidney disease, and to establish the relationship between these parameters and markers of the functional state of the kidneys.

Material and methods

Experimental animals

For the experiment, 40 male Wistar rats aged 5-6 weeks and weighing between 250-270 g were obtained from the Institute of Pharmacology and Toxicology of Academy of Medical Sciences of Ukraine. All stages of the research were carried out following the rules for humane treatment of experimental animals, approved by Committee on Bioethics of Vinnytsya National Pirogov Memorial Medical University, Ukraine, and international animal welfare rules which agreed with the regulations of the European Convention for Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The animals were identified using a system of individual colored labels on the body; seasonal and circadian animal rhythms were taken into account throughout the study. Throughout the experiments, animals stayed under the standard environmental conditions (the temperature was 22.5±1.0°C, relative humidity: 55–65%, and dark cycle: 12 h light/12 h dark) with a one-week adaptation before the experiment. They were housed in cages made of polypropylene and had free access to food and water *ad libitum.*

Surgical procedures, CKD modeling and animal grouping

Chronic kidney disease (CKD) was induced by a two-step procedure. First, a total resection of the left kidney with 5/6 nephrectomy of the contralateral kidney was performed [18]. The rats were anesthetized by

intraperitoneal injection of 5% ketamine (2 ml/kg) and fixed in the right supine position. After shaving and disinfection, a 2 cm-long skin incision perpendicular to the left side of the spine was made. Then, the muscles were cut and the whole right kidney was removed by direct surgical excision. The muscles and skin were sutured, followed by disinfection of the surgical incision. After one week, the second surgery was performed. The right kidney was gradually exposed out of the body surface. After separating the renal capsule, 2/3 branches of the right renal artery were ligated. The injured kidney tissue surface was immediately compressed with the hemostatic sponge to stop bleeding. The remnant kidney was placed in the abdomen, and then the muscles and skin were sutured, followed by disinfection of the surgical incision. The biochemical indexes of rats were measured after 4 weeks.

Rats were divided into two groups. Group 1 (n=20) consisted of sham-operated (control) rats. Sham-operated controls undergo the same procedure to expose the kidneys. Instead of extirpating the kidney or cutting the poles one week later, both kidneys were decapsulated at a one-week interval taking care not to disturb the adrenal glands. Group 2 (n=20) consisted of 5/6 nephrectomized rats. All animals were examined after 45 days after the first operation.

Biochemical and functional measurements

Homogenates and post-nuclear supernatant of remnant kidneys were evaluated by biochemical studies. The kidneys were perfused with a cold 1.15% solution of potassium chloride, shredded with scissors, and homogenized in a 1.15% potassium chloride medium in a 1:3 ratio (mass/volume) at 3000 rpm (Teflon/glass). The post-nuclear fraction was obtained by centrifugation of homogenates for 30 min at 600 g and +4°C. The aliquots of post-nuclear supernatant were collected in Eppendorf microtubes and stored at -20°C for analysis.

The H_2S content in the post-nuclear supernatant was determined using a spectrophotometric method by reaction with N, N-dimethyl-para-phenylenediamine in the presence of $FeCl_3$ [19]. The activity of H_2S-synthesizing enzymes – cystathionine gamma-lyase (CSE, EC 4.4.1.1), cystathionine beta-synthase (CBS, EC 4.2.1.22), and cysteine aminotransferase (CAT, EC 2.6.1.3) was evaluated according to the growth of a sulfide anion in the incubation medium as adapted by our group [20]. Concentrations of substrates and cofactors, pH, and duration of incubation provided optimal conditions for determining the activity of the enzymes selected *a priori*. The incubation medium contained pyridine oxalphosphate 0.67 mmol, L-cysteine 3.3 mmol, and Tris-buffer 0.083 M (pH 8.5) (final concentrations). 0.5 ml of the incubation medium was introduced into the tubes and 0.1 ml of samples containing 1-2 mg of the protein from the supernatant were added. To prevent loss of H_2S, the tubes were covered with a "Parafilm" film and incubated at 37°C. Control samples were incubated without homogenate, which was added only after the reaction had been stopped. The reaction was stopped by cooling the test tubes on ice, after which 1% zinc acetate solution was added to bind the sulfide anion, plus a 20 mM solution of N, N-dimethyl-para-phenylenediamine in 7.2 mMHCl, and a 30 mM solution of $FeCl_3$ in 1.2M HCl. The tubes were incubated for 20 min at 18-25°C, then 20% trichloroacetic acid was added and the samples were centrifuged for 10 min at 1500 g. The optical density of the supernatant was measured by a photoelectric calorimeter at a wavelength of 670 nm. Control samples were treated as experimental samples, except that the test material was introduced into the medium after incubation and cooling. The H_2S content in the medium was determined as mentioned above [21]. The amount of H_2S formed was calculated using a standard sample with 0.1 ml of a 312 mM $Na_2S \times 9H_2O$ solution instead of the supernatant.

The ability of kidneys to utilize exogenous H_2S was determined by the rate of decrease in the concentration of sulfide anion in the incubation medium [22]. 0.1 ml of a post-nuclear supernatant of kidney homogenate was added to 0.1 ml of the incubation medium containing 312 μM Na_2S, 0.47 mM Tris-HCl buffer (pH 7.4) (final concentrations) and incubated for 30 min at 37°C in sterile, sealed plastic Eppendorf tubes. Control samples were incubated without homogenate, which was added only after stopping the reaction. The reaction was stopped by cooling the test tubes on ice, after which 0.5 ml of 1% zinc acetate solution was added to the sulfide anion and its amount was determined by the methylene blue reaction using established methods [21].

Urinary excretion of the protein serum creatinine (Cr) was monitored after water load (5% of body mass). The content of creatinine in blood serum and urine was determined by the Jaffe method using standard kits from Filicit-Diagnostics-Diagnostics, Ukraine. Creatinine clearance, glomerular filtration rate (GFR, ml/min), and reabsorption of water (%) were calculated according to the established formulas:

$$\text{GFR (ml/min)} = \frac{\text{Urine creatinine (µmol/L)}}{\text{Plasma creatinine (µmol/L)}} \cdot \text{Urine flow rate (ml/min)}$$

$$\text{Reabsorption of water (\%)} = \frac{\text{GFR (ml/min)} - \text{Urine flow rate (ml/min)}}{\text{GFR (ml/min)}} \cdot 100\ \%$$

The content of sodium and potassium in serum and urine was determined by a spectrophotometric method according to the standard set from Filicit-Diagnostics-Diagnostics, Ukraine. The protein level was determined by the microbio ureteric method with Benedict's reagent.

Statistical analysis

The data were analyzed by ANOVA (analysis of variance) followed by Dunnett's test (Statistical Package for Social Sciences, SPSS 17.0, USA). All quantitative data were expressed as the mean ± standard error (M±S.E.M.). Since all data fulfilled the criteria for normal distribution by the Kolmogorov-Smirnov test, further analysis was performed using the parametric Student's t-test. Pearson's correlation coefficient was calculated to investigate the relationship between parameters. The difference was statistically significant if $p<0.05$.

Results

Experimental chronic renal failure (CKD) in rats is accompanied by a decrease in H_2S content in the kidneys (Figure 1). Thus, in the sham-operated animals' group, the median content of H_2S in the kidneys was 3.73 nmol/mg of protein, whereas the level of H_2S in the kidneys in the CKD group was decreased by 35.80% ($p<0.05$) compared to the control group (Figure 1). These results may have been attributed to an increase in the rate of H_2S utilization in non-enzymatic oxidation reactions and/or a decrease in enzymatic formation of H_2S in reactions catalyzed by CSE, CBS, and CAT in the kidney. The activity of H_2S-producing enzymes in the kidney and the activity of H_2S utilization in animals with CKD were next assessed.

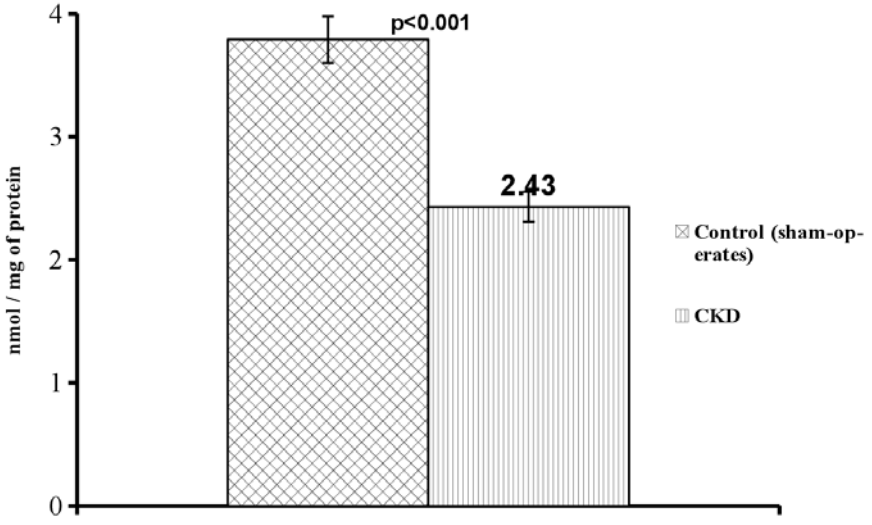

Figure 1. H_2S content in kidney of rats with CKD
Note: Data are mean ± SEM, n=10.

Animals with experimental CKD had a statistically significant decrease in enzymatic production of H_2S in the kidneys (Figure 2). CSE-induced formation of H_2S through hydrolytic cleavage of cysteine, CBS-induced H_2S synthesis through the condensation reaction of cysteine with homocysteine, and CAT-induced H_2S production through cysteine transamination by α-ketoglutarate were decreased by 28.3%, 30.2%, and 34.2%, respectively, in rats with CKD compared to sham-operated animals.

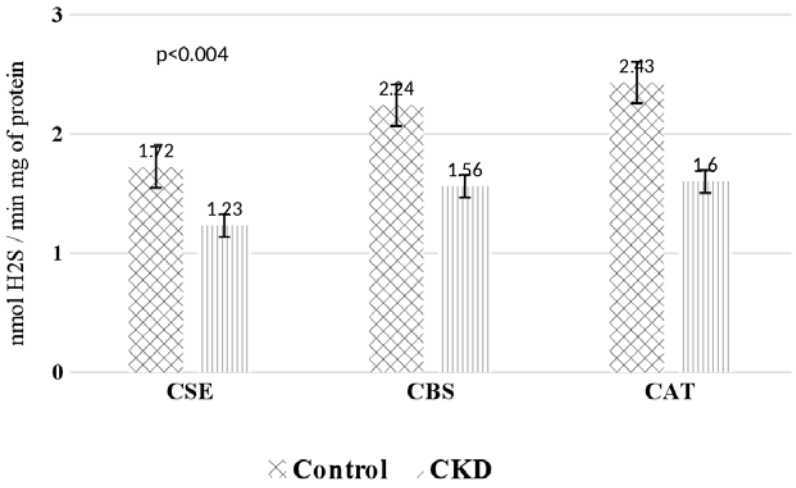

Figure 2. The activity of H_2S-producing enzymes in kidney of rats with CKD
Note: Data are mean ± SEM, n=10; p – differences versus control (sham-operated) group; CSE – cystathionine gamma-lyase; CBS – cystathionine beta-synthase; CAT – cysteine aminotransferase.

Investigation of non-enzymatic utilization of exogenous H_2S in rats' kidneys showed that CKD was associated an acceleration in H_2S oxidative degradation (Figure 3). In the CKD animals, the rate of exogenous H_2S utilization in the kidney was 34.3% higher ($p<0.05$) than in the control.

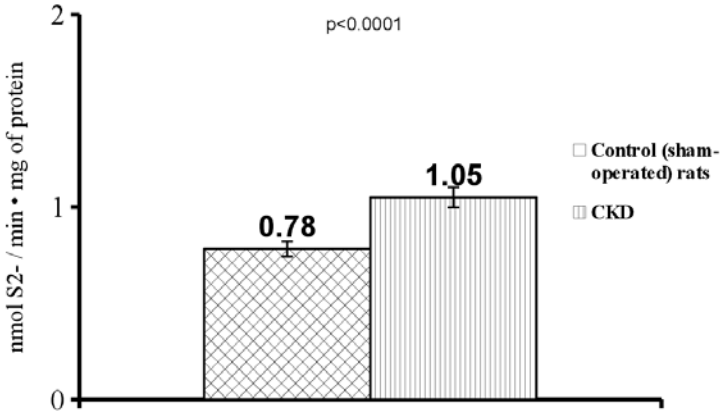

Figure 3. Speed of exogenous H_2S utilization in kidney of rats with CKD
Note: Data are mean ± SEM, n=10.

The next step was to determine to what extent the changes in the levels of H_2S were associated with the disruption of renal functions. CKD animals exhibited a 51.2% increase ($p<0.05$) in the content of creatinine in blood plasma, and a 30.6% decrease ($p<0.05$) in creatinine levels in the urine compared with sham-operated animals (Table 1).

Table 1. Changes in renal function in rats with experimental CKD in comparison with control (sham-operated) animals

Experimental groups	Control (sham-operated)	CKD	p (ANOVA)
Diuresis, ml/8 hr	5.38±0.13	3.50±0.14	p<0.001
Plasma creatinine, µmol/L	86.0±2.45	130±4.36	p<0.0001
Urine creatinine, µmol/L	7.07±0.19	4.91±0.18	p<0.0001
GFR, ml/min	0.461±0.011	0.139±0.008	p<0.0001
Na (urine, µmol/8 h)	2.40±0.12	1.66±0.08	p<0.004
K (urine, µmol/8 h)	38.2±1.06	13.8±0.74	p<0.001
Na / K	0.063±0.002	0.122±0.006	p<0.001

Na (blood plasma, mmol/L)	142±4.07	196±3.11	p<0.001
K (blood plasma, mmol/L)	4.60±0.14	9.68±0.17	p<0.004
Protein (urine, mg/8 hr)	0.855±0.011	1.45±0.003	p<0.0001
Reabsorption of water, %	97.6±0.05	94.7±0.19	p<0.0001

Note: The results are expressed as mean ± standard error.

Experimental renal failure in rats was accompanied by a significant decrease in diuresis and glomerular filtration rate (GFR). In the control group, diuresis was within 4.60-5.70 ml/8 h, and the GFR was 0.415-0.512 ml/min. In contrast, diuresis was reduced by 34.9% ($p<0.05$), while GFR was reduced by 69.9% ($p<0.05$) in animals with CKD compared to the sham-operated animals.

A correlation test showed that diuresis and GFR rates likely depend on the H_2S concentration in rat kidneys. There was a significant direct correlation between the H_2S level in kidneys and diuresis ($r=0.75$; $p<0.05$). An even greater correlation was found between the H_2S level in kidneys and GFR ($r=0.85$; $p<0.05$). These results suggest that the decrease in H_2S levels in the kidney was associated with an increase in dysfunction of the excretory organs.

We evaluated the changes in electrolyte derangements in rats with CKD and their association with the H_2S level in rat kidneys by examining the effect of CKD on sodium metabolism in rats (sodium concentration in blood plasma and its excretion with urine). The experimental renal pathology in rats resulted in retention of sodium in the blood and impaired elimination of sodium with the urine. Sodium concentration in the plasma was 37.9% higher ($p<0.05$), while excretion of sodium with the urine was 30.8% lower ($p<0.05$) in CKD animals compared with the control group.

We found sodium metabolism was closely associated with H_2S metabolism in CKD animals, as evidenced by the results of a correlation analysis. There was a significant correlation between H_2S levels in the kidney and the sodium concentration in blood ($r=-0.66$; $p<0.05$), while urinary excretion of sodium directly correlated with the level of H_2S in the kidneys ($r=0.63$; $p<0.05$).

Along with sodium metabolism disorders, chronic renal insufficiency is accompanied by derangements of potassium metabolism in rats. Hyperkalemia is one of the most common and life-threatening electrolyte disorders in CKD and becomes increasingly prevalent as CKD advances. We found that chronic kidney pathology in rats caused an imbalance in potassium elimination, which is manifested as an increase in potassium levels in plasma and a reduction in urinary excretion. In animals with renal insufficiency, the concentration of potassium in blood plasma was 110% higher ($p<0.05$), and excretion with urine was 63.9% lower ($p<0.05$), compared with the control group. In animals with underlying CKD, a reduction in potassium elimination was associated with a deficiency in H_2S production in the kidneys, as shown by correlation analysis. There was a significant negative correlation between H_2S level in the kidneys and the concentration of potassium in blood ($r=-0.69$; $p<0.05$), and a positive correlation between H_2S levels and excretion of potassium in urine ($r=0.65$; $p<0.05$).

We estimated the relative ratio of sodium and potassium excreted in the urine, which is one of the markers of the regulatory effect of the aldosterone system on kidney function. Experimental renal failure was accompanied by an increase in the Na/K ratio in rat urine. In the group of rats with CKD, the average ratio of Na/K in urine was higher by 94.7% ($p<0.05$) compared with sham-operated animals. The deficiency in the production of H_2S in the kidneys of rats with underlying CKD was an important factor causing a disturbance in the regulation of renal function by aldosterone. There was a significant negative correlation between the H_2S level in kidneys and the Na/K ratio in urine ($r=-0.59$; $p<0.05$).

CKD caused a disruption in water reabsorption in nephron tubules, which was confirmed by a decrease in the water reabsorption coefficient. In the control group, the median water reabsorption rate was 97.6%, whereas in animals with CKD exhibited a reduced water reabsorption rate. This indicator positively correlated with H_2S content in rat kidneys ($r=0.54$; $p<0.05$).

Another confirmation of tubular dysfunction in CKD animals was the development of proteinuria. In the control group, proteinuria was 0.852 mg/8 h, whereas in the experimental pathology condition proteinuria increased by 69.6% ($p<0.05$). One of the causes of tubular dysfunction and proteinuria in rats with CKD was a deficiency in H_2S production in the kidneys of rats. Similar to previous experiments, H_2S content in the kidneys of rats negatively correlated with urinary protein excretion ($r=-0.58$; $p<0.05$).

Discussion

We determined that rats with CKD exhibited large-scale disruption of H_2S metabolism in the kidneys. The enzymatic formation of H_2S in reactions catalyzed by CSE, CBS, and CAT was reduced, while the rate of

non-enzymatic oxidative degradation increased, which was accompanied by a decrease in H_2S in the kidneys. A deficiency in H_2S production was associated with disruptions in kidney function in rats with the experimental pathology, namely, oliguria, reduction of glomerular filtration rate, creatinine elimination disturbance, metabolic derangements of sodium and potassium, and tubular disorders (reduction of water reabsorption and proteinuria).

Our further studies have shown that modulators of H_2S metabolism caused a multidirectional effect on glomerular and tubular kidney functions in rats with CKD. In particular, the administration of propargylglycine (the CSE-inhibitor) resulted in a decrease in H_2S levels in kidneys, which was accompanied by an intensification of glomerular and tubular dysfunction. For example, GFR significantly decreased by 44.4%, while proteinuria significantly increased by 29.7%. At the same time, the introduction of a donor of H_2S (sodium hydrogen sulfide) restored the levels of H_2S in the kidneys and produced a nephro-protective effect, including improved kidney filtration function, water reabsorption, electrolyte exchange, and tubular apparatus state. For example, GFR significantly increased by 102%, while proteinuria significantly decreased by 24.1% (Data not published). These studies indicate the important role of the H_2S system in the regulation of renal function in normal rats and rats with CKD.

There remain a number of questions regarding the molecular mechanisms by which H_2S deficiency in the kidneys contributes to the development of filtration and tubular dysfunction in response to chronic renal failure. It is known that H_2S is an important antioxidant and cytoprotector, and therefore its deficiency in kidneys may play a role in activating free radical oxidation processes involving lipids and proteins, leading to damage of glomerular and tubular cells [23]. Thus, H_2S is a known anti-apoptotic factor, and its absence can induce apoptosis and tissue damage in the glomerular and tubular kidney systems [24-26].

H_2S is a major factor in hyperpolarization and vascular relaxation due to its ability to activate ATP sensitive K+-channels in endothelium and smooth muscle due to their S-sulfhydration; in addition, H_2S can stimulate endogenous nitrogen production of monoxide [26,27]. The depressive effect of CKD on the H_2S system may be one of the pathogenetic mechanisms driving renal blood supply disruption.

Many studies have shown that H_2S and its precursors, sodium hydrogen sulfide and cysteine, can stimulate filtration in kidneys through activation of K+ATP- channels in afferent arterioles of glomeruli, and also contributes to the excretion of potassium and sodium ions by inhibition of the Na+/K+/2Cl- cotransporter and the Na+/K+ -ATPase in tubule cells [28]. Therefore, it is clear that a H_2S deficit is an important factor in the development of filtration deficiencies and disruptions in the reabsorption of electrolytes in nephron tubules.

Another possible mechanism for nephrotoxicity in response to reduced H_2S levels may be hyperactivation of the renin-angiotensin-aldosterone system, since H_2S can reduce the expression of renin and its release from kidney cells in the juxtaglomerular kidney apparatus [29].

Taking into account the experimental nature of this work and the use of a limited range of studies, it can be assumed that there is a link between disorders of H_2S metabolism in the kidneys and the development of CKD in animals. However, further in-depth experimental studies of the effects of various H_2S inducers, synthesis activators, and H_2S donors, as well as inhibitors of enzyme systems that mediate H_2S synthesis, on the molecular mechanisms of kidney damage (oxidative stress, nitrogen monoxide system, renin-angiotensin system, apoptosis, etc.) will determine the pathogenetic links in the development of CKD associated with impaired H_2S metabolism. A promising area is to conduct clinical population studies using multivariate regression analysis, which will establish the contribution of disruption of the H_2S system in the development of CKD.

Our data suggests that the H_2S system in kidneys is an important metabolic target, which can influence the efficacy of treatments to improve the functional state of kidneys in CKD.

Conclusions

CKD in rats is accompanied by an imbalance of hydrogen sulfide (H_2S) metabolism in rat kidneys: activity of H_2S-producing enzymes decreases by 28.3-34.2% ($p<0.05$), the rate of exogenous H_2S utilization in kidneys increases by 34.3% ($p<0.05$), and H_2S content is reduced by 35.8% ($p<0.05$) compared to control animals.

In comparison with the control group, CKD animals exhibit an elevation (by 30-70%, $p<0.05$) of sodium and potassium concentration in the blood and elimination of protein in the urine, as well as a decrease (in 1.4-2.1 folds, $p<0.05$) of diuresis, GFR, and sodium and potassium excretion. In addition, glomerulotubular disturbances in CKD are positively correlated with H_2S content in the kidneys ($r=0.54-0.85$, $p<0.05$).

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