Role of Nitric Oxide Pathway in Development and Progression of Chronic Kidney Disease in Rats Sensitive and Resistant to its Occurrence in an Experimental Model of 5/6 Nephrectomy

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1,2   **Marek Saracyn**
BDE 3   **Katarzyna Czarzasta**
ACE 4   **Marek Brytan**
CD 5   **Piotr Murawski**
CEF 6   **Sławomir Lewicki**
BEF 7   **Tomasz Ząbkowski**
CDE 6   **Robert Zdanowski**
ACD 2   **Agnieszka Cudnoch-Jędrzejewska**
ADG 1   **Grzegorz Witkor Kamiński**
ACDE 2   **Zofia Wańkowicz**

1 Department of Endocrinology and Isotope Therapy, Military Institute of Medicine, Warsaw, Poland
2 Department of Internal Diseases, Nephrology, and Dialysis, Military Institute of Medicine, Warsaw, Poland
3 Department of Experimental and Clinical Physiology, Laboratory Centre for Preclinical Research, Medical University of Warsaw, Warsaw, Poland
4 Department of Pharmacology and Toxicology, Military Institute of Hygiene and Epidemiology, Warsaw, Poland
5 Department of Information Technology, Military Institute of Medicine, Warsaw, Poland
6 Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Warsaw, Poland
7 Department of Urology, Military Institute of Medicine, Warsaw, Poland

Corresponding Author: Marek Saracyn, e-mail: msaracyn@interia.pl
Source of support: The study was supported by grant No. 216/KBL/12 from the Ministry of Science and Higher Education

Background: Understanding the mechanisms conditioning development of chronic kidney disease (CKD) is still a challenge. The aim of this study was to evaluate the activity of the intrarenal nitric oxide (NO) pathway in the context of sensitivity or resistance of different animal strains to the development and degree of renal failure.

Material/Methods: Two rat strains were used: Wistar (WR) and Sprague-Dawley rats (SDR) in a model of CKD – 5/6 nephrectomy. We assessed parameters of renal failure and expression of nitric oxide synthase (NOS) isoforms in renal cortex and medulla.

Results: We did not observe renal failure in WR, and CKD developed in SDR with increase of creatinine and urea concentration as well as decrease of diuresis and glomerular filtration. In the renal cortex, baseline expression of NOS2 was higher in WR than in SDR. 5/6 nephrectomy resulted in reduction of NOS2 in both strains and NOS3 in WR. In the renal medulla, baseline NOS2 expression was higher in SDR, and nephrectomy resulted in its decrease only in SDR. Although baseline NOS3 expression was higher in SDR, the NOS3 expression after nephrectomy was higher in WR rats.

Conclusions: In model of CKD – 5/6 nephrectomy, SDR proved to be sensitive and WR resistant to development of CKD. The intrarenal activity of the nitric oxide pathway was the factor that differentiated both strains. This mechanism may be responsible for insensitivity of WR to development of renal failure in this model of CKD.

MeSH Keywords: Models, Animal • Nitric Oxide • Rats, Sprague-Dawley • Rats, Wistar • Renal Insufficiency, Chronic

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/903820
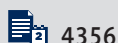
📄 4356   ▦ 1   📊 2   📖 26

Background

Chronic kidney disease (CKD) is an increasingly serious clinical problem. It is estimated that worldwide there are over 600 million patients at different stages of CKD [1,2]. Diabetes, hypertension, and primary renal diseases, including mainly chronic glomerulonephritis, remain the most common causes of CKD [3]. Multifactorial pathogenesis, different picture, and lack of effective therapy, as well as chronic and progressive nature of the disease stimulate and focus new research on attempts to slow the progression of CKD [4]. Clearly, the greatest interest of researchers concerns congenital endogenous factors and nephroprotective mechanisms [5]. The main question is: How does the same causative factor in one patient result in a slight and transient worsening of renal function, and in another patient result in severe and sometimes irreversible organ failure? Detailed knowledge of such mechanisms would allow the early prevention of kidney disease and reduce the effect on the rate of development, disease severity, and its prognosis.

To the best of our knowledge, we were the first to report that the strain of experimental animals in a model of acute renal failure (ARF) determines the sensitivity or resistance to the development and the degree of renal failure [6,7]. In contrast to Sprague-Dawley rats (SDR), which developed acute renal failure in our study in a conventional manner, Wistar rats remained completely resistant to the development of renal failure, despite the use of Gal at the same or twice the dose used in SDR. This appears to be the first observation that, in this experimental model, some inherited genetic predispositions were responsible for the development and extent of acute renal failure. In subsequent studies in the same experimental model of acute renal failure, we observed that the manipulation of the activity of endogenous nitric oxide, both in the preventive and therapeutic manner, was a factor affecting the value of diuresis, natriuresis, urine osmolality, osmotic clearance, and free water clearance, and determined the degree of renal failure [8].

The problem of sensitivity of different strains of experimental animals to renal failure development was also discussed in other experimental models. Erderly et al. observed a significant resistance of Wistar Furth rats to the development and progression of renal failure in several experimental models of CKD, in comparison to other animal strains [9,10]. These authors found that the number of active nephrons in Wistar Furth rats was 35% higher than in Sprague-Dawley rats. In addition, the total basal production of endogenous nitric oxide and that stimulated by kidney damage was significantly higher in these animals. In the present study, we decided to verify our own findings and those available in the literature about whether the renal activity of endogenous nitric oxide is responsible for the sensitivity of experimental animals to the development and degree of renal failure in an experimental model of CKD, 5/6 nephrectomy.

The aim of our study was to assess the activity of intrarenal nitric oxide pathway in the context of the sensitivity or resistance of 2 rat strains – Wistar and Sprague-Dawley – and its influence on the development and degree of renal failure in an experimental model of CKD, 5/6 nephrectomy.

Material and Methods

Animals

Our study was approved by the 2nd Local Ethics Committee for Experiments on Animals at Warsaw Medical University (decision number 18/2015). The study included 32 randomly selected animals: 16 male Sprague-Dawley and 16 male Wistar rats. 10-week-old Wistar rats were obtained from the Department of Laboratory Animals of the Polish Mother's Memorial Hospital Research Institute, Łódź, Poland. 10-week-old Sprague-Dawley rats were obtained from the Central Laboratory of Experimental Animals, Medical University of Warsaw, Warsaw, Poland. All animals were kept in standard cages, fed a standard diet, and had free access to water and food. They were all maintained under a natural 12-h diurnal day/night cycle, at a constant temperature of 22±2°C and constant humidity of 45–50%. The experiments were performed between 10: 00 and 18: 00 h on naturally moving animals performing normal activities. During the experiments, the animals were placed individually in metabolic cages with free access to food and water. All experiments were performed in accordance with the principles of the Scientific Animals Procedures Act of 1986.

Experimental protocols

The animals were randomly divided into 4 groups (*n*=8 in each): the sham Wistar group (Group 1); the tested Wistar group (Group 2) with 5/6 renal mass reduction; the sham Sprague-Dawley group (Group 3); and the tested Sprague-Dawley group (Group 4) with 5/6 renal mass reduction.

The 5/6 renal mass reduction model of CKD

The 5/6 nephrectomy (5/6 NX) as a model of CKD was performed on 10-week-old rats under general anesthesia (intraperitoneal injection of ketamine 10 mg/100 g body weight, and intraperitoneal injection of xylazine 1 mg/100 g body weight). An incision in the skin in the lumbar area on the left, parallel to the spine, was made after immobilization of the animal in prone position. The left kidney was removed in its entirety after ligation of vessels and ureter. Two weeks after the first intervention, a second operation was performed. In the prone position, the skin was cut in the lumbar region on the right, parallel to the spine, and the right kidney was recovered. After ligation, the top and bottom poles (superior and interior

segments) were removed. The wound was closed by surgical sutures (Vicryl 6.0, Ethicon).

Sham surgery (NLK)

In animals treated with sham nephrectomy, the left kidney was removed and the right was gently touched by the needle. The wound was closed by surgical sutures (Vicryl 6.0, Ethicon). At the end of both surgical procedures, the animals were given an analgesic (intraperitoneal injection of buprenorphine chloride 3 g/100 g body weight, 5.95 nmol/ml twice daily for 2–3 days) and an antibiotic (intramuscular injection of penicillin, Polfa 10 000 IU/100 g body weight, 0.047 mmol/ml).

Collection of blood

Eight weeks after the second surgery, 6-ml blood samples were collected from the beating heart of the animals under deep general anesthesia.

Collection of urine

We collected 24-h urine collection was performed 8 weeks after the second surgery and analyzed it immediately after collection.

Collection of the kidneys for RT PCR analysis

After collecting the blood and the urine, the animals were euthanized by intraperitoneal injection of a lethal dose of ketamine to collect the kidney cortex and the kidney medulla. The fragments of organs were placed in liquid nitrogen and then stored in a freezer (–80°C) until analysis.

Biochemical blood and urine tests

Biochemistry was performed in serum or urine using an Integra 700 autoanalyzer (Roche, USA), and reagents for the determination of sodium, potassium, creatinine, and urea were obtained from Roche (Germany). An automatic osmometer (Knauer, Germany) was used to measure the osmolality of the urine and serum. Urine specific gravity was measured with a Junior II Miditron analyser (Roche Diagnostics, France), and reagents were purchased from Roche Diagnostics (France). Proteinuria was measured daily using an AU 680 analyser (Beckman Coulter, USA), and reagents for the determination of proteinuria were purchased from Beckman Coulter (USA).

Calculation of renal parameters

Creatinine clearance as a parameter of glomerular filtration was calculated according to the following formula: creatinine clearance (creat cl) (ml/min)=$(creat_u \times V_{U\,24\,h})/(creat_s \times 1440)$, where $creat_u$ is the creatinine concentration in urine, $creat_s$ is

the creatinine concentration in blood serum, and $V_{U\,24\,h}$ is the daily urine volume. Osmolality clearance was calculated according to the following formula: osmolality clearance (osmol cl) (ml/24 h)=$(U\,osm \times V_{U\,24\,h})/P\,osm$, where U osm is the osmolality of the urine, $V_{U\,24\,h}$ is the daily urine volume, and P osm is the plasma osmolality. The plasma osmolality was calculated according to the following formula: $P\,osm=2 \times (Na_s+K_s+urea_s)$, where Na_s is the sodium concentration in the blood serum, K_s is the potassium concentration in the blood serum, and $urea_s$ is the urea concentration in the blood serum. Free water clearance was calculated according to the following formula: free water clearance (FW cl) (ml/24 h)=$V_{U\,24\,h}$–osmol cl, where $V_{U\,24\,h}$ is the daily urine volume, and osmol cl is the osmolality clearance. The excreted fraction of sodium was calculated according to the following formula: fractional sodium excretion (FE_{Na}%) (%)–$(Na_u/Na_s)*(creat_u \backslash creat_s)*100\%$, where Na_u is the sodium concentration in urine, Na_s is the sodium concentration in blood serum, $creat_u$ is the creatinine concentration in urine, and $creat_s$ is the creatinine concentration in blood serum. Free water reabsorption was calculated according to the following formula: free water reabsorption (FW reab) (ml/24 h) =osmol cl–$V_{U\,24\,h}$, where osmol cl is the osmolality clearance, and $V_{U\,24\,h}$ is the daily urine volume [11].

mRNA expression of NOS2 and NOS3 (real-time PCR)

The fragments of collected organs were homogenized in TRIzol® reagent (Ambion, Life Technologies) using a TissuLyser LT homogenizer (Qiagen). Subsequently, mRNA was extracted using the PureLink RNA Mini Kit (Ambion, Life Technologies). Then, multiplex reactions were carried out. The reaction mixture contained TaqMan RNA-to-CT 1-Step Kit, primers for the gene of interest (NOS2 and NOS3; Applied Biosystems) labelled with FAM reporter dye, primers for GAPDH (Applied Biosystems) labelled with VIC, RNA, and RNase-free water (Life Technologies). The final volume of reaction mixture (50 µl) was subjected to proliferation under conditions: 15 s at 95°C and 1 min at 60°C for 40 cycles in a ViiA™ 7 Real-Time PCR System thermocycler (Applied Biosystems). The relative gene expression was given, on the basis of estimations of the values of the delta cycle threshold (ΔCt), as relative amounts to the endogenous control.

Statistical analysis

Statistical analyses were performed using Statistica 12 software, with the Student's t-test and an analysis of variance when multiple comparisons were needed. Where necessary, the Mann-Whitney U test was applied for the analysis of nonparametric data. A p value <0.05 was assumed as the level of significance. All data are expressed as means ± standard deviation.

Results

Renal parameters in Wistar rats before and after 5/6 nephrectomy

Table 1 shows the results of Wistar rats renal parameters before (group 1 – WR control group) and 8 weeks after 5/6 nephrectomy (group 2–5/6Nx). We did not observe progression of renal failure in Wistar rats after 5/6 nephrectomy in comparison to control animals. Serum creatinine, urea, potassium, and urine creatinine, sodium, and potassium did not change significantly in either group. Moreover, plasma osmolality, daily natriuresis, sodium ejected fraction, and proteinuria also did not differ significantly. Although daily diuresis and glomerular filtration rate decreased slightly in the group that underwent nephrectomy compared with the control group, the difference was statistically insignificant. Only urine osmolality (p-0.007), osmotic clearance (p-0.001), free water reabsorption (p-0.0006), and urine specific gravity (p-0.01) were lower in the 5/6Nx group. Other renal parameters and clearance parameters evaluating kidney function in both groups of Wistar rats are shown in Table 1.

Renal parameters in Sprague-Dawley rats before and after 5/6 nephrectomy

Table 1 also contains renal profile of Sprague-Dawley rats before (group 3 – SDR control group) and after the 5/6 nephrectomy. In contrast to Wistar rats, Sprague-Dawley rats developed full-blown chronic renal failure 8 weeks after surgery. In comparison to control animals, 5/6 nephrectomized rats demonstrated significantly increased serum concentrations of creatinine (p-0.0008) and urea (p-0.0001), and plasma osmolality (p-0.0001), and a significant decrease of urea concentration in urine (p 0.03), daily natriuresis (p-0.006) and sodium ejected fraction (p-0.006). Furthermore, the value of the glomerular filtration rate and daily diuresis were significantly reduced (p-0.004 and p-0.0009). In addition, significant reduction was found of the osmotic clearance (p-0.0005), free water reabsorption (p-0.0005), and daily proteinuria (p-0.01). Other renal parameters and clearance indices evaluating kidney function in both groups of Sprague-Dawley rats are shown in Table 1.

Wistar and Sprague-Dawley rats and renal failure

Direct comparison of Wistar and Sprague-Dawley rats proved that the 2 strains differed significantly in the tested profile of renal indices, both in basal conditions (control group) and stimulated conditions, after exposure to nephrectomy (5/6 Nx groups). SDR rats, as compared to WR, were initially characterized by a significantly higher daily diuresis (p-0.0002) and natriuresis (p-0.0005), higher daily proteinuria (p-0.01), lower urine osmolality (p-0.00001), and lower urine specific gravity

(p-0.0002). Moreover, we found lower levels of creatinine (p 0.02) and urea (p-0.0001) in urine in SDR compared to WR rats. In contrast, serum creatinine and potassium as well as GFR and plasma osmolality did not differ significantly.

The differences were more pronounced in both groups (5/6 Nx WR *vs.* 5/6 Nx SDR) under stimulated conditions after kidney damage. In SDR, as compared to WR, we recorded significantly higher levels of serum creatinine (p-0.0006) and urea (p-0.0001), and plasma osmolality (p-0.0002), and significantly lower glomerular filtration rate. Furthermore, in SDR compared to WR, we observed significantly lower levels of urine creatinine (p-0.01) and urea (p-0.0001), and lower values of urine specific gravity, higher levels of sodium (p-0.01) in urine, and higher values of daily natriuresis (p-0.008). Other renal and clearance indices of the 2 rat strains are shown in Table 1.

Expression of NOS2 and NOS3 mRNA in the renal cortex of WR and SDR

We recorded significant differences in the level of NOS2 mRNA in the renal cortex of WR and SDR rats between the tested groups (Figure 1A). Both in WR and SDR, 5/6 nephrectomy resulted in a statistically significant reduction in NOS2 mRNA (p-0.013 and p-0.0037, respectively). Moreover, the initial NOS2 mRNA level in the basal conditions was significantly higher in WR than in SDR. As regards NOS3, we observed significant differences only in WR rats (Figure 1B). Reducing the kidney mass by 5/6 in this animal strain caused a significant reduction in NOS3 mRNA expression (p-0.011).

Expression of NOS2 and NOS3 mRNA in the renal medulla of WR and SDR

The relationships of NOS2 and NOS3 transcript levels in the renal medulla of WR and SDR rats were different between the studied groups. In the case of NOS2, mRNA expression of this protein in the basal conditions was significantly higher in SDR than in WR (p-0.033) and 5/6 nephrectomy resulted in a significant reduction in the NOS2 transcript level only in SDR (Figure 2A). For NOS3, nephrectomy resulted in a significant increase in the mRNA expression level of this protein only in WR (p-0.0002). Although NOS3 transcript level in the basal conditions was higher in SDR (p-0.006), mRNA expression level of this protein in stimulated conditions, following the 5/6 reduction of the renal mass, was significantly higher in WR rats (p-0.038) (Figure 2B).

Discussion

In our previous studies, we have found that the animal strain determined the development and degree of renal failure in a

Table 1. Renal parameters in Wistar and Sparague-Dawley rats.

Gr. (n)	$Creat_s$ mg/dl	$Urea_s$ mg/dl	Creat cl ml/min	Na_s mmol/l	K_s mmol/l	$Osmol_p$ mosmol/kg	$Creat_u$ mg/dl	$Urea_u$ mg/dl	Na_u mmol/l	K_u mmol/l	Na_u 24 h mmol/24 h
1 (12) sham WR	0.45± 0.07	44± 5.9	0.48± 0.11	140.3± 1.9	5.8± 0.3	307.1± 5.7	65.4± 5.9	7040.2± 278.9	30.5± 7.7	271.8± 31.8	0.14± 0.04
2 (12) 5/6 Nx WR	0.44± 0.04	40.8± 8.1	0.39± 0.14	143.5± 2.2	5.9± 0.5	312.5± 5.6	61.3± 17.5	5076± 1087.2	30.3± 6.9	242.2± 53.1	0.12± 0.03
3 (12) sham SDR	0.5± 0.05	35± 3.3	0.83± 0.5	142.1± 1.1	5.7± 0.3	307.4± 2.2	51.6± 13.1	3355.5± 857.7	53.21± 15.4	186.2± 35.4	0.59± 0.27
4 (12) 5/6 Nx SDR	0.72± 0.1	76.6± 8.1	0.19± 0.11	142.8± 1.2	6.1± 0.3	323.6± 2.3	35.5± 16.8	2151.8± 1015.7	43.7± 10.8	77.3± 21.1	0.25± 0.11
Gr. 2 vs. Gr. 1 – p	ns	ns	ns	0.01*	ns	ns	ns	0.003**	ns	ns	ns
Gr. 4 vs. Gr. 3 – p	0.0008***	0.0001***	0.004**	ns	ns	0.0001***	ns	0.03*	ns	0.006**	0.006**
Gr. 3 vs. Gr. 1 – p	ns	0.003**	ns	ns	ns	ns	0.02*	0.0001***	0.003**	0.003**	0.0005***
Gr. 4 vs. Gr.2 – p	0.0006***	0.0001***	0.01*	ns	ns	0.0002***	0.01*	0.0001***	0.01*	0.0002***	0.008**

Gr. (n)	FE_{Na}%	$Osmol_u$ mosmol/kg	Osmol cl ml/24 h	FW cl ml/24 h	Osm_u/osm_p	$Creat_u$/$creat_s$	FW reab ml/24 h	SG_u	$Protein_u$ (mg/dl)	$Protein_u$ 24 h mg/24 h	24 h urine ml/24 h
1 (8) sham WR	0.32± 0.11	1796± 29.1	27.3± 2.6	-22.6± 2.3	5.7± 0.2	149.5± 28.6	22.6± 2.3	1.02± 0.001	200.6± 14.8	9.4± 1.4	4.6± 0.4
2 (8) 5/6 Nx WR	0.31± 0.12	1496± 254.9	19.1± 4.5	-15.1± 4	4.7± 0.7	142.1± 46.5	15.1± 4	1.01± 0.005	165.5± 52.9	6.7± 2.4	4.1± 0.8
3 (8) sham SDR	0.41± 0.18	1506.5± 66.8	52.9± 12.5	-42.1± 10.1	4.9± 0.2	106.5± 38.5	42.1± 10.1	1.006± 0.004	188.2± 62.3	21.5± 12.5	10.8± 2.6
4 (8) 5/6 Nx SDR	0.15± 0.08	1455.7± 100.8	25.9± 10.1	-20.2± 8.2	4.4± 0.3	48.8± 21.2	20.2± 8.2	1.004± 0.006	131.2± 58.1	7.5± 5.1	5.6± 1.9
Gr. 2 vs. Gr. 1 – p	ns	0.007**	0.001**	0.006**	0.005**	ns	0.0006***	0.01*	ns	0.01*	ns
Gr. 4 vs. Gr. 3 – p	0.006**	ns	0.0005***	0.0005***	0.01*	0.003**	0.0005***	ns	ns	0.01*	0.0009***
Gr. 3 vs. Gr. 1 – p	ns	0.00001***	0.0001***	0.0001***	0.00001***	0.03*	0.0001***	0.0002***	ns	0.01*	0.0002***
Gr. 4 vs. Gr. 2 – p	0.01*	ns	ns	ns	ns	0.0002***	ns	0.003**	ns	ns	ns

* p<0.05; ** p<0.01; *** p<0.001. 24 h urine – twenty-four-hour urine collection; $creat_s$ – serum creatinine; $urea_s$ – serum urea; creat cl – creatinine clearance; Na_s – serum sodium; K_s – serum potassium; $osmol_p$ – plasma osmolality; $creat_u$ – urine creatinine; $urea_u$ – urine urea; Na_u – urine sodium; K_u – urine potassium, $Na_{u\ 24h}$ – 24h urine sodium excretion, $osmol_u$ – urine osmolality, osmol cl – osmolal clearance, FW cl – free water clearance, FE_{Na}% – ejected fraction of sodium, osm_u/osm_p – urine osmolality/plasma osmolality ratio, $creat_u$/$creat_s$ – urine creatinine/serum creatinine ratio, FW reab – free water reabsorption, SG_u – specific gravity of urine, $protein_u$ – concentration of protein in urine, $protein_{u\ 24h}$ – 24h proteinuria, p – value of p. Biochemical parameters were evaluated 8 weeks after surgery. 24h urine samples were collected 8 weeks after surgery. Values are means ±SE, significance – p<0.05. Group 1 – sham Wistar, group 2–5/6 nephrectomy Wistar, group 3 – sham Sprague-Dawley, group 4–5/6 nephrectomy Sprague-Dawley.

model of acute renal failure [6,7]. Sprague-Dawley rats developed typical acute renal failure, while Wistar rats were completely resistant to the development of acute organ failure. In the present study, we have demonstrated that in the experimental model of chronic renal failure – 5/6 nephrectomy – Sprague-Dawley strain rats were sensitive, while Wistar strain rats were resistant to the development of chronic renal failure when the same damaging agent was applied in both strains (the reduction of kidney mass by 5/6).

The impact of congenital genetic predispositions on kidney function in different experimental models was also observed by other authors. Erdely et al. demonstrated results similar to our findings in other models of CKD [9,10,12]. In their studies, Wistar Furth rats, in contrast to Sprague-Dawley rats, proved to be sensitive to the development of renal failure in a model of CKD induced by the administration of mineralocorticosteroid, aminoglycoside, or partial renal ablation. Kidney damage as a result of unilateral nephrectomy combined with the
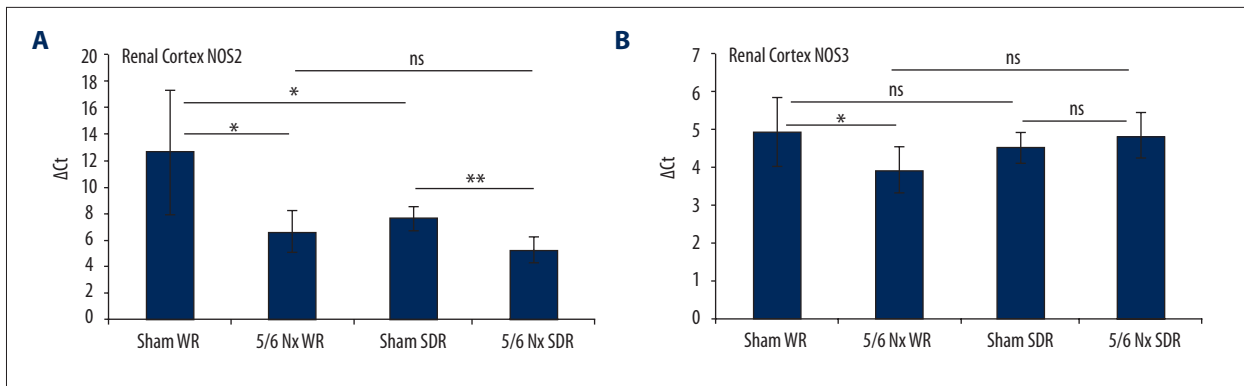
Figure 1. (**A**) Expression of NOS2 mRNA in kidney cortex in sham WR, sham SDR, and 5/6 Nx WR, 5/6 Nx SDR. NOS – nitric oxide synthase, WR – Wistar rats, SDR – Sprague-Dawley rats, 5/6 Nx – 5/6 nephrectomy, * p<0.05, ** p<0.01, ns – not significant, values are means ±SD as delta cycle threshold (ΔCt) as relative amounts to the endogenous control. (**B**) Expression of NOS3 mRNA in kidney cortex in sham WR, sham SDR and 5/6 Nx WR and 5/6 Nx SDR. NOS – nitric oxide synthase, WR – Wistar rats, SDR – Sprague-Dawley rats, 5/6 Nx – 5/6 nephrectomy, * p<0.05, ns – not significant, values are means ±SD as delta cycle threshold ΔCt) as relative amounts to the endogenous control.
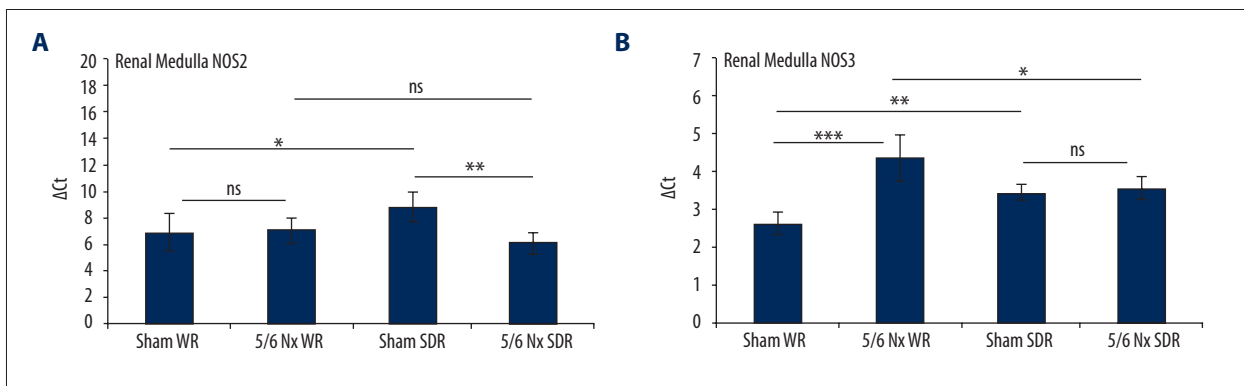
Figure 2. (**A**) Expression of NOS2 mRNA in kidney medulla in sham WR, sham SDR, 5/6 Nx WR, and 5/6 Nx SDR. NOS – nitric oxide synthase, WR – Wistar rats, SDR – Sprague-Dawley rats, 5/6 Nx – 5/6 nephrectomy, * p<0.05, ** p<0.01, ns – not significant, values are means ±SD as delta cycle threshold (ΔCt) as relative amounts to the endogenous control. (**B**) Expression of NOS3 mRNA in kidney medulla in sham WR, sham SDR, 5/6 Nx WR, and 5/6 Nx SDR. NOS – nitric oxide synthase, WR – Wistar rats, SDR – Sprague-Dawley rats, 5/6 Nx – 5/6 nephrectomy, * p<0.05, ** p<0.01, *** p<0.001, ns – not significant, values are means ±SD as delta cycle threshold (ΔCt) as relative amounts to the endogenous control.

administration of deoxycorticosterone and salt, as well as repeated administration of puromycin or partial ablation of 1 kidney connected with the nephrectomy of the other, did not significantly deteriorate renal function in Wistar Furth rats, but significantly decreased GFR in Sprague-Dawley rats.

Similar dependencies were also observed in other strains. Basile et al. observed that Brown-Norway (BN) rats, compared to Sprague-Dawley rats, in a model of acute renal failure caused by 45-min ischemia with subsequent reperfusion of the kidney (ischemia/reperfusion model of ARF) remained completely insensitive to the acute damage of the kidneys [13]. BN rats exposed to bilateral kidney ischemia/reperfusion after 24 h demonstrated no significant differences in the concentration of creatinine, urine output, or urine sodium excretion compared to the control group. In contrast, SD rats developed acute organ failure 24 h after kidney damage, with a significant increase in serum creatinine, diuresis, and natriuresis. At 48 h after ischemia, SDR presented a typical image of tubular necrosis. In BN rats, in contrast to SDR, renal histology showed no significant changes.

Kökény et al. studied the susceptibility of different experimental animal strains to the development of renal fibrosis [14]. In one of their studies, they showed that, in a model of unilateral nephrectomy combined with partial ablation/infarction of the other kidney, kidney fibrosis was much more advanced in SD rats 5 weeks after surgery compared with Rowett rats. The degree of fibrosis expressed as staining intensity of fibronectin and TGF-β1 was significantly higher in Rowett rats compared to SDR, both within glomeruli and renal interstitium.

Slightly different results were reported in another experimental model of renal toxicity induced with acetaminophen and p-aminophenol. Tarlof et al. found that, in contrast to Fischer 334 rats (F334), SDR were completely insensitive to toxic damage of the kidneys after administration of acetaminophen [15]. In contrast, the nephrotoxic effect of aminophenol was similar in both strains of animals.

What are the factors and mechanisms that determine these different effects of the same factors in damaging the kidneys in the same species of experimental animals, when the only difference is that the animals are of different strains? In our previous studies, in an acute renal failure model induced by Gal, we observed that the manipulation of the activity of endogenous nitric oxide in both preventive and therapeutic manner in the same experimental model of acute renal failure was a factor affecting the value of diuresis, natriuresis, urine osmolality, osmotic clearance, free water clearance, and glomerular filtration rate, and finally determined the degree of renal failure [8]. Inhibition of endogenous NO, in dose-dependent manner of NOS inhibitor, increased daily sodium excretion and daily diuresis. Furthermore, it also improved glomerular filtration. Prophylactic use of the inhibitor was in this case more effective than its application after Gal administration. On the other hand, stimulation of the NO system, regardless of the NO donor dose applied, more distinctly increased daily diuresis and natriuresis after administration of Gal. Furthermore, activation of the NO system after Gal administration in a dose-dependent manner positively affected the value of glomerular filtration.

Nitric oxide (NO) has many functions in living organisms that are responsible for a variety of biological effects in the central and peripheral nervous system, as well as in the immune and cardiovascular systems [16]. NO is not stored in the cellular structures, and its synthesis is carried out on demand. There are no cellular mechanisms that regulate the intracellular concentration of NO; therefore, regulation of its biological activity is determined by the availability of substrate and the presence of synthesis inhibitors [17]. NO is derived from the amino acid L-arginine (L-Arg) with the participation of nitric oxide synthase (NOS) [18]. Three different isoforms of this enzyme have been identified: neuronal (nNOS-type I, constitutive), inducible (iNOS-Type II, non-constitutive), and endothelial (eNOS-type III, constitutive) [19,20]. nNOS-type I is activated by depolarization of the axonal ends in the central and peripheral nervous system. Synthesis of iNOS-Type II occurs under the influence of de novo inflammatory cytokines, bacterial endotoxins, or lipopolysaccharides in the cells of the immune system. eNOS-type III is an enzyme specific to the tunica intima of vascular walls, and its synthesis is enhanced by all phenomena changing the tension of this layer [21]. Nitric oxide, synthesized with the involvement of NOS, acts through secondary messengers, including cyclic GMP (cGMP) and G

kinase or by a cGMP-independent cascade involving certain ion channels, enzymes, and other effector proteins [22,23]. One such biological effect is vasodilation and improvement of renal blood flow.

In the present study, we observed that the initial NOS2 mRNA expression level in the renal cortex in basal conditions was significantly higher in WR than in SDR. No such differences were recorded for NOS3. NOS2 expression in the renal medulla under basal conditions was indeed increased in SDR, but 5/6 nephrectomy reduced the mRNA level of this protein only in this strain. The analysis of NOS3 expression in the renal medulla provided the most interesting results. Although NOS3 transcript level in the basal conditions was higher in SDR, mRNA expression level of this protein in stimulated conditions, following the 5/6 reduction of the kidney mass, was significantly higher in WR rats. This mechanism, in our opinion, may be responsible or co-responsible for the insensitivity of WR to the development of renal failure in this experimental model.

Results partially consistent with our observations were presented by Erdely et al. in the above-cited papers. In the CKD model induced by deoxycorticosterone and excess salt (DOCA/salt-induced CKD), the total renal activity of NO and baseline and stimulated NOS1 density in the kidney cortex and medulla was higher 5 weeks after kidney damage in WR compared to SDR [9]. Interestingly, NOS3 density was comparable in both strains. In another model of CKD induced by puromycin, the same authors showed that, although the total baseline renal activity of NOS was higher in SDR after puromycin organ damage, the total renal NOS activity was higher in WR, both in the cortex and medulla of the kidney [10]. Moreover, NOS1 density in WR was higher in the renal cortex before and after organ injury, but was higher in the medulla only after damage. The density of NOS3, as in the previous model, was comparable in both strains. In another model of CKD – unilateral nephrectomy combined with a partial ablation of the other kidney – the same authors showed that the baseline total cortical activity of NOS was higher in SDR as compared to WR, and this activity was significantly higher in the WR strain after the renal damage [12]. Furthermore, both the baseline and stimulated NOS1 density in the renal cortex and stimulated in the renal medulla were significantly higher in WR than in SDR. Moreover, administration of non-specific NOS inhibitor after renal damage caused exacerbation of renal failure, proving a significant involvement of the endogenous NO pathway in CKD progression. Moningka et al., in a model of acute kidney injury after unilateral nephrectomy and 35 min of ischemia/reperfusion (ischemia/reperfusion AKI model) of the second kidney, observed that spontaneous physical activity of SDR reduced the density of NOS3 in the renal cortex at the baseline and, to a greater extent, after a 6-week training [24]. In Fischer 334 (F334) rats, physical activity significantly increased the density of NOS3 in

the renal cortex. Moreover, physical activity exacerbated renal failure in SDR after kidney damage, while in F334 rats, the degree of renal failure remained unchanged.

Our study is partially consistent with and complementary to the studies discussed above. Firstly, we used a different experimental model of CKD than in the studies cited above. Secondly, we evaluated the expression of NOS2, which was not done in most of those studies, and thirdly, we evaluated mRNA expression level of different NOS isoforms. Most importantly, in the tested animal strains we have shown differences in NOS3 expression, a constitutive isoform of NOS, the activity of which plays a central role in the regulation of vascular tension, blood flow, and, ultimately, renal perfusion.

The role of NOS2 in this respect seems also special. Thus far, NOS2 has been postulated to be primarily activated by inflammatory mediators and subsequently involved in inducing apoptosis, as well as processes leading to cell necrosis [16]. The presence of inducible NOS isoform (NOS2) has been confirmed in healthy kidneys, even though it was not a constitutive isoform [19]. Moreover, our study showed that after 5/6 nephrectomy, NOS2 expression was significantly lower in the damaged kidney and in chronic renal failure. Furthermore, higher NOS2 activity, both at baseline and in stimulated conditions, was found in WR animals (in the strain resistant to development of renal failure). This suggests a different, nephroprotective role, of the NOS2-activated NO pathway. A similar observation was reported by Chu et al. in acute thioacetamide-induced liver failure [25], reporting that the selective blockade of NOS2 compared with simultaneous inhibition of both isoforms of NOS (NOS2 and NOS3) had beneficial effects on the mortality of experimental animals and all markers of liver failure. On the other hand, NO has strong antioxidant properties,

operates as a free radical scavenger, inhibits lipid peroxidation, and has a protective effect on many substances in nitrosylation processes [20]. In a study by Fiorucci et al., activation of the NO pathway prevented changes in mitochondrial membrane polarization and the transfer of mitochondrial enzymes into the cytosol, thereby inhibiting mechanisms leading to damage and cellular apoptosis [26]. Perhaps the higher activity of the NO pathway through NOS2 activation in the renal cortex of WR rats is responsible for the observed resistance to development of renal failure in our experimental model. This new insight into the role of NOS2 activity in strain-dependent renal failure undoubtedly requires further research.

Conclusions

Our study has demonstrated that in the 5/6 nephrectomy experimental model of chronic kidney disease, Sprague-Dawley rats are sensitive to development of chronic renal failure, but Wistar rats are resistant. The intrarenal activity of the nitric oxide pathway, to a different extent in the cortex and medulla, was the factor that differentiated the 2 strains. NOS2 expression in the renal cortex and NOS3 in the renal medulla were significantly higher in Wistar rats. This mechanism may be responsible for the insensitivity of this strain to the development of renal failure in this experimental model. Further experimental studies should evaluate the participation of other metabolic pathways that differentiate these 2 strains in terms of their sensitivity to the development of acute or chronic renal failure. Future clinical studies should also determine whether this mechanism of risk of chronic kidney diseases might be implemented in humans. Expression of NOSs in kidney tissues evaluated, for instance, during diagnostic renal biopsy, might be worth exploring.

References:

1. Dienemann T, Fujii N, Orlandi P et al: International Network of Chronic Kidney Disease cohort studies (iNET-CKD): A global network of chronic kidney disease cohorts. BMC Nephrol, 2016; 17: 121–30

2. Niemczyk S, Niemczyk L, Szamotulska K et al: Is free testosterone concentration a prognostic factor of survival in chronic renal failure (CRF)? Med Sci Monit, 2015; 21: 3401–8

3. Fischer MJ, Hsu JY, Lora CM et al: CKD progression and mortality among Hispanics and non-Hispanics. J Am Soc Nephrol, 2016; 27: 3488–97

4. Jingxian F, Peng Y, Yingdeng W et al: Prevalence and clinical significance of low T3 syndrome in non-dialysis patients with chronic kidney disease. Med Sci Monit, 2016; 22: 1171–79

5. Parsa A, Kanetsky PA, Xiao R: Genome-wide association of CKD progression: The chronic renal insufficiency cohort study. J Am Soc Nephrol, 2017; 28(3): 923–34

6. Saracyn M, Pirożyńska E, Kozłowski W, Wańkowicz Z: If the race of experimental animals might be the main factor responsible for development of renal failure in animal model of hepatorenal syndrome (HRS)? Nephrol Dial Transplant, 2003; 18: 605-W184. Proceedings of the World Congress of Nephrology, 2003 Jun 8–12, Berlin

7. Saracyn M, Patera J, Kocik J et al: Strain of experimental animals and modulation of nitric oxide pathway: Their influence on development of renal failure in an experimental model of hepatorenal syndrome. Arch Med Sci, 2012; 8: 555–62

8. Saracyn M, Ząbkowski T, Zdanowski R et al: Effect of nitric oxide pathway regulation on water/sodium balance and renal function in a rodent model of acute liver and renal failure. Med Sci Monit, 2014; 20: 1735–44

9. Erdely A, Freshour G, Tain Y-L et al: DOCA/NaCl-induced chronic kidney disease: A comparison of renal nitric oxide production in resistant and susceptible rat strains. Am J Physiol Renal Physiol, 2007; 292: F192–96

10. Erdely A, Freshour G, Smith C et al: Protection against puromycin aminonucleoside-induced chronic renal disease in the Wistar-Furth rat. Am J Physiol Renal Physiol, 2004; 287: F81–89

11. Anand R, Harry D, Holt S et al: Endothelin is an important determinant of renal function in a rat model of acute liver and renal failure. Gut, 2002; 50: 111–17

12. Erdely A, Wagner L, Muller V et al: Protection of Wistar Furth rats from chronic renal disease is associated with maintained renal nitric oxide synthase. J Am Soc Nephrol, 2003; 14: 2526–33

13. Basile DP, Donohoe D, Cao X, Van Why SK: Resistance to ischemic acute renal failure in the Brown Norway rat: A new model to study cytoprotection. Kidney Int, 2004; 65: 2201–11

14. Kökény G, Németh Z, Godó M, Hamar P: The Rowett rat strain is resistant to renal fibrosis. Nephrol Dial Transplant, 2010; 25: 1458–62

15. Tarloff JB, Goldstein RS, Morgan DG, Hook JB: Acetaminophen and p-aminophenol nephrotoxicity in aging male Sprague-Dawley and Fisher 344 rats. Fundam Appl Toxicol, 1989; 12: 78–91

16. Loscalzo J: The identification of nitric oxide as endothelium-derived relaxing factor. Circ Res, 2013; 113: 100–3

17. Hu LS, George J, Wang JH: Current concepts on the role of nitric oxide in portal hypertension. World J Gastroenterol, 2013; 19: 1707–17

18. Klinger JR, Abman SH, Gladwin MT: Nitric oxide deficiency and endothelial dysfunction in pulmonary arterial hypertension. Am J Respir Crit Care Med, 2013; 188: 639–46

19. Huang H, Silverman RB: Recent advances toward improving the bioavailability of neuronal nitric oxide synthase inhibitors. Curr Top Med Chem, 2013; 13: 803–12

20. Carnicer R, Crabtree MJ, Sivakumaran V et al: Nitric oxide synthases in heart failure. Antioxid Redox Signal, 2013; 18: 1078–99

21. McNaughton L, Puttagunta L, Martinez-Cuesta MA et al: Distribution of nitric oxide synthase in normal and cirrhotic human liver. Proc Natl Acad Sci USA, 2002; 99: 17161–66

22. Wang JH, Redmond HP, Wu QD, Bouchier-Hayes D: Nitric oxide mediates hepatocyte injury. Am J Physiol, 1998; 275: G1117–26

23. Jiang W, Desjardins P, Butterworth RF: Minocycline attenuates oxidative/nitrosative stress and cerebral complications of acute liver failure in rats. Neurochem Int, 2009; 55: 601–5

24. Moningka NC, Cunningham MW Jr., Sterling M et al: Effects of voluntary wheel running on the kidney at baseline and after ischaemia-reperfusion-induced acute kidney injury: a strain difference comparison. J Physiol, 2013; 591: 1313–24

25. Chu CJ, Chang CC, Wang TF et al: Detrimental effects of nitric oxide inhibition on hepatic encephalopathy in rats with thioacetamide-induced fulminant hepatic failure: Role of nitric oxide synthase isoforms. J Gastroenterol Hepatol, 2006; 21: 1194–99

26. Fiorucci S, Antonelli E, Distrutti E et al: Liver delivery of NO by NCX-1000 protects against acute liver failure and mitochondrial dysfunction induced by APAP in mice. Br J Pharmacol, 2004; 143: 33–42