Peptides Restore Functional State of the Kidneys during Cisplatin-Induced Acute Renal Failure

I. I. Zamorskii, T. S. Shchudrova, N. S. Lin'kova,***, T. E. Nichik***, and V. Kh. Khavinson*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 6, pp. 708-713, June, 2015
Original article submitted July 10, 2014

The effects of polypeptide complex from the kidney and short peptides AED, EDL, and AEDG on renal functions were studied in rats with cisplatin-induced acute renal failure. AED peptide decreased protein excretion and electrolyte concentration in the urine. Polypeptide complex from the kidney and peptides EDL and AEDG normalized diuresis, creatinine concentration in the urine and its excretion, glomerular filtration rate, and absolute resorption of sodium ions and reduced protein concentration in the urine and its excretion, the concentrations of sodium and potassium ions in the urine, and other parameters. EDL peptide produced was potent nephroprotective effect. It is known that the polypeptide complex of the kidney and short peptides restore the expression of signal molecules (marker of functional state of the kidneys), so these peptide substances can have nephroprotective effect during various renal pathologies.

Key Words: *acute renal failure; cisplatin; peptides*

Renal pathology ranks eleventh among the states impairing quality of life and increasing mortality in humans of different age groups. In 40% patients, acute renal failure (ARF) develops in advanced age [4]. In 20-30% cases, ARF is caused by toxic agents, including 18-27% caused by pharmacotherapy side effects [5,11]. ARF develops in 20-30% of patients treated with cisplatin, an antineoplastic with pronounced nephrotoxicity [11,12]. In epithelial cells of S3 segment of renal proximal tubules, cisplatin disintegrates to its nephrotoxic metabolites. Its concentration in the urine 5-fold surpasses that in the blood plasma. The antitumor and toxic effects of cisplatin are determined by damage to mitochondrial and to in a lower extent nuclear DNA, which explains high sensitivity of epithelial cells in the renal proximal tubules enriched with mitochondria to this drug [11,12]. Cisplatin disturbs mitochondrial function, which leads to inhibition ATPases and transport systems, changes cation concentrations in cells, ROS generation, and progressive ATP deficit. Primary and secondary damage to the renal tubules suppresses of tubular function and leads to of electrolyte loss. Other mechanisms of cisplatin nephrotoxicity also are oxidative stress, inflammation, fibrogenesis, and activation of necrosis by high doses or induction of mitochondrion-mediated apoptosis of cells in the renal tubules by lower doses of the agent [11-14].

For solving the problems of nephrology and gerontology, in particular, the search of new effective and safe methods of ARF treatment of in elderly and senile patients, new peptide bioregulators exhibiting kidney-protective properties were developed in the St. Petersburg Institute of Bioregulation and Gerontology [2,9]. Polypeptide complex from the kidneys (PCK) was efficient on the model of experimental nephrolithiasis in animals (where it reduced the concentration of oxalate-ions in the urine, the level of calcium deposits in the renal tissue, and intensity of free radical processes) and in patients with renal pathologies [2].

Bukovinian State Medical University, Chernovtsy, Ukraine; *I. I. Mechnikov North-Western State Medical University; **St. Petersburg State Polytechnic University; ***St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia. ***Address for correspondence:*** tshchudrova@gmail.com T. S. Shchudrova; linkova@gerontology.ru. N. S. Lin'kova

0007-4888/15/1596-0736 © 2015 Springer Science+Business Media New York

PCK and peptides AED and EDL stimulate the growth of organotypic cell cultures of the kidneys from young and old animals. In primary dissociated cultures of renal cells, PCK and peptides AED and EDL regulated the expression of cell renewal markers (Ki-67, and p53), intracellular matrix remodeling markers (MMP-14), and IL-8 synthesis during cell aging [7]. Recent experiments showed that PCK and peptides AED and EDL activate cell growth by suppressing the expression of aging markers p16, p21, and p53 and increasing of expression of SIRT-6 protein (reduced synthesis of SIRT-6 is a marker of aging in cultures renal cells) [4,7]. According to theoretical and experimental data, molecular models of AED and EDL interaction with various DNA sites were proposed. It was hypothesized that both peptides form the most energetically effective complexes with $d(ATATATATAT)_2$ sequence in the minor groove of DNA. It was found that binding of peptides AED and EDL with this sequence can affect the expression of genes encoding aging markers in renal cells [4,7,9]. However, the nephroprotective effects of short peptides AED and EDL in experimental ARF were never studied. Peptide AEDG structurally similar to AED demonstrates antioxidant, anti-inflammatory, immunomodulating, and regulatory properties, which makes it a promising agent for targeting the main mechanisms of renal failure. In addition, AEDG protein stimulates secretion of melatonin that exhibits nephroprotective properties [10]. Nephroprotective activity of AEDG protein in rhabdomyolytic ARF has been previously demonstrated [15].

Here we studied the effects of PCK and peptides AED, EDL, and AEDG on excretory and ion-regulating functions of the kidneys during the development of cisplatin-induced ARF.

MATERIALS AND METHODS

Experiments were performed on 3-month-old outbred albino rats (n=42) weighting 120-200g kept under standard vivarium conditions with constant temperature and humidity and free access to water and food (complete food for laboratory animals, Kormotech). The animals were divided into 6 equal groups using the method of stratification randomizing: intact rats (group 1; sacrificed at the same time as treatment group rats for measuring of control values of study parameters); rats with cisplatin-induced ARF (group 2); animals with cisplatin-induced ARF receiving PCK in a dose of 300 μg/kg (group 3); animals with cisplatin-induced ARF receiving EDL peptide in a dose of 3 μg/kg (group 4); rats with cisplatin-induced ARF receiving AED peptide in a dose of 3 μg/kg (group 5); animals with cisplatin-induced ARF receiving AEDG peptide in a dose of 7 μg/kg (group 6).

According to standard methods of modeling of renal disorders in pharmacological studies [8,13], cisplatin-induced ARF was modeled by a single intraperitoneal administration of cisplatin (EBEWE Pharma) in a dose of 6 mg/kg 72 h before sacrifice. The test peptides were intraperitoneally injected to animals of groups 3-6 for 4 days before and 3 days after cisplatin treatment once a day in the morning. Doses of peptides were calculated from the recommended doses for human per body weight of animals using a coefficient of species specificity. Peptide solutions were prepared before the beginning of experimental series by dissolving in necessary volume of water for injections.

The urine was collected over 2 h in 72 h after cisplatin treatment under the conditions of forced dieresis (parenteral administration of warm water (37°C) via a gastric tube in a volume of 5% body weight). The animals were sacrificed by decapitation under ether narcosis in accordance to the Rules of European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Excretory function of the kidneys was estimated using parameters of diuresis, glomerular filtration rate (GFR), concentration of creatinine in blood plasma and urine, and concentration and excretion of protein with the urine. Creatinine concentration in blood plasma was measured using Popper method modified by Merzon, and in the urine using Folin method. Protein concentration in the urine was measured by sulphosalicylic method [3]. The ion-regulating function of the kidneys was evaluated by urinary concentration and excretion of sodium ions, reabsorption, proximal and distal transport, and clearance of sodium ions, and urinary concentration and excretion potassium ions [6]. Concentrations of potassium and sodium ions in blood plasma and urine were measured by flame photometry using a FPL-1 photometer [3]. The parameters of renal function were standardized per body weight or volume of glomerular filtrate.

Statistical analysis of the data was performed using SPSS Statistics 17.0 software. The type of distribution was estimated using Kolmogorov–Smirnov criterion. Estimation of the differences between the samples was conducted using parametric Student's t test (for normal distribution) and nonparametric Mann–Whitney U test (for distribution not conforming the normal law). The level of significance was $p \leq 0.05$.

RESULTS

The development of cisplatin-induced ARF was followed by significant changes in the functional state of rat kidneys. Primary toxic injury to the proximal tubules was accompanied by suppression of the transport systems in cells, which led to disturbances in

TABLE 1. Effects of Peptides on Functional State of the Kidneys under Conditions of Cisplatin-Induced ARF in Rats (*M*±*m*)

Parameters	Control (group 1)	ARF (group 2)	ARF+PCK (group 3)	ARF+peptide EDL (group 4)	ARF+peptide AED (group 5)	ARF+peptide AEDG (group 6)
Diuresis, ml	4.12±0.17	1.50±0.25**	2.62±0.31++	3.02±0.51++	1.81±0.14	2.89±0.28++
Creatinin concentration in blood plasma, μmol/liter	64.90±3.91	94.91±12.92*	67.43±9.29+	68.95±5.13+	82.60±5.89	77.54±10.44
Creatinin excretion, μmol/2 h	3.83±0.17	2.45±0.34*	3.31±0.35+	4.26±0.48++	2.94±0.25	3.88±0.28++
GFR, μl/min	502.90±37.82	240.8±46.88**	433.3±41.43++	543.9±89.68++	305.1±33.08	469.9±70.93+
Protein concentration in the urine, g/liter	0.011±0.002	0.049±0.007**	0.020±0.002++	0.026±0.003++	0.025±0.002++	0.026±0.003++
Protein excretion, mg/100 μl	0.009±0.002	0.037±0.012**	0.013±0.002++	0.014±0.002++	0.015±0.001++	0.019±0.005
Na^+ concentration in the urine, μmol/liter	0.63±0.05	2.94±0.16**	0.85±0.10++	0.79±0.55++	1.47±0.11++	1.20±0.18++
Na^+ excretion, μmol/100 μl GF	0.54±0.08	1.94±0.23**	0.53±0.07++	0.45±0.14++	1.45±0.38	0.93±0.25+
Absolute resorption of Na^+, μmol/min	69.69±10.66	26.38±5.37*	45.09±3.95+	57.13±9.49+	32.60±3.30	51.05±8.28+
Proximal transport of Na^+, μmol/2 h	7.33±0.65	3.00±0.63**	5.13±0.47+	6.54±1.09+	3.72±0.39	5.81±0.97+
Distal transport of Na^+, μmol/2 h	533.87±22.38	162.6±28.5**	276.2±36.2+	316.2±52.7+	192.3±13.7	314.9±35.4++
K^+ concentration in blood plasms, μmol/liter	5.29±0.28	4.11±0.21**	4.39±0.24	5.71±0.32++	5.68±0.29++	5.11±0.27++
K^+ excretion, μmol/100 μl GF	3.97±0.49	21.25±4.55**	12.49±1.68	9.94±0.97++	14.52±1.23	15.22±3.71

Note. GF, glomerular filtrate. *$p \le 0.05$, **$p \le 0.01$ in comparison with control; +$p \le 0.05$, ++$p \le 0.05$ in comparison with ARF.

reabsorption of sodium and potassium ions in the proximal and distal tubules and increased excretion of these ions (Table 1). The urinary concentration and excretion of sodium ions increased by 4.6 and 3.5 times, respectively. Absolute resorption of sodium ions decreased by 2.6 times, which can be related to suppression of proximal transport by 2.4 times and distal transport by 3.2 times. It is known that increased NaCl concentration near the *macula densa* leads to an increase in vascular resistance via tubule-glomerular feedback and can result in a decrease of renal blood flow followed by a decrease in GFR [1]. This effect was observed in group 2 animals: diuresis decreased by 2.6 times with the development of oliguric stage of ARF; GFR and creatinine excretion decreased by 2.2 and 1.5 times, respectively, which was accompanied by an increase in creatinine concentration in blood plasma and indicated the development of retention azotemia. Significant proteinuria was found in animals with ARF: protein concentration in the urine increased by 4.5 times and its excretion by 4 times. The development of ARF was accompanied by a 5.3-fold increase in potassium excretion, which can be related to enhanced effects of aldosterone on cells of collecting tubules due to activation of the renin-angiotensin-aldosterone system [1]. Under these conditions, the concentration of potassium ions in blood plasma decreased by 1.3 times, which indicated the development of hypokalemia, a typical adverse effect of cisplatin treatment [11,12,14].

Treatment of ARF with the test peptide bioregulators led to improvement of functional state of the kidneys. Administration of PCK, EDL , and AEDG to animals with ARF increased dieresis by 1.7, 2, and 1.9 times, respectively, which can be explained by GFR increase and attests to prevention of oliguria and better prognosis for these animals in comparison with non-treated rats. Creatinine secretion significantly increased after injection of PCK, EDL, and AEDG by 1.3, 1.7, and 1.6 times, respectively, in comparison with ARF group, which was followed by a decrease in hyperazotemia intensity. All test peptides reduced protein concentration in the urine (PCK, by 2.4 times; EDL, by 1.9 times; AED, by 2 times; AEDG, by 1.9 times). In addition, all peptides decreased protein excretion (PCK, by 2.8 times; EDL, by 2.6 times; AED, by 2.5 times; AEDG, by 1.9 times).

Excretion of sodium ions with the urine also decreased after treatment with the peptides (PCK, by 3.7 times; EDL, by 4.3 times; AEDG, by 2.1 times, in comparison with ARF group). Reduced loss of ions with the urine is related to the protective effects of the proteins on tubular cells. This suggestion is confirmed by improvement of processes of reabsorption of sodium ions after administration of PCK (by 1.7 times), EDL (by 2.2 times), and AEDG (by 1.9 times). Treatment with PCK and AEDG significantly increased the parameters of proximal and distal transport of sodium ions (by 1.7 and 1.9 times, respectively), which indicates the preserving of mechanisms of calcium balance in the tubules. EDL also enhanced proximal and distal transport of sodium ions by 2.2 and 1.9 tines, respectively. Excretion of potassium ions significantly decreased by 2.1 times after EDL injection. All test oligopeptides normalized the level of potassium ions in the blood and prevented the development of hypokalemia.

Administration of PCK and short peptides AED, EDL, and AEDG in preventive and treatment regimens improved the functional state of rat kidneys under conditions of cisplatin-induced ARF. AED promoted reduction of concentrations of protein and sodium and potassium ions in the urine. PCK, EDL, and AEDG normalized the studied parameters of kidney functions. EDL produced maximum nephroprotective effect.

Thus, PCK is an effective substance for the treatment of experimental ARF in animals, which is coincident with previous data on its usage in patients with gouty nephropathy. In 78% of cases, treatment with PCK was followed by improvement of the results of blood and urine tests, blood biochemistry, and positive dynamics of kidney state during ultrasound investigation [2]. The short peptides, especially EDL, produced significant nephroprotective effect during experimental cisplatin-induced ARF. These data are very important, as short peptide exhibit low immunogenity in comparison with polypeptide complexes and can be potential drugs for patients with impaired immune system function. It has been previously demonstrated that mechanisms of effects of study peptides are based on their ability to regulate synthesis of proteins contributing to the processes of cell regeneration and slowing-down aging processes in renal tissue [7,9]. Thus, obtained results show new perspectives for investigation of PCK and peptides AED, EDL, and AEDG as nephroprtective agents during various renal pathologies.

REFERENCES

1. A. I. Gozhenko, *Patologiya*, **5**, No. 3, 66-75 (2008).
2. V. V. Khavinson, V. V. Malinin, and G. A. Ryzhak, *Eurasian Patent No. 010723*, *Substance Normalizing Renal Function and Method of Its Synthesis*, October 30, 2008.
3. V. S. Kamyshnikova, *Manual for Clinical and Biochemical Analysis and Laboratory Diagnostics* [in Russian], Moscow (2009).
4. T. E. Nichuk, N. S. Lin'kova, N. A. Kraskovskaya, *et al*., *Uspekhi Fiziol. Nauk*, **45**, No. 2, 49-56 (2014).
5. O. O. Petyuk, N. I. Voloshchuk, and O. V. Mashevs'ka, *Rats. Farmakoter*., No. 1, 6-15 (2009).
6. S. I. Ryabov and Yu. V. Natochkin, *Functional Neurology* [in Russian], St. Petersburg (1997).
7. V. Kh. Khavintson, N. S. Lin'kova, V. O. Polyakova, *et al*., *Bull. Exp. Biol. Med.*, **157**, No. 2, 261-264 (2014).
8. S. Yu. Schtrigol', V. M. Lisovii, and I. A. Zupanen', *Methods for Experimental Modeling of Kidney Failure in Pharmacological Studies* [in Ukrainian], Kiev (2009).
9. V. Kh. Khavinson, N. S. Linkova, A. V. Trofimov, *et al*., *Biol. Bull. Rev*., **1**, No. 4, 389-393 (2011).
10. U. Kilic, E. Kilic, Z. Tuzcu, *et al*., *Nutr. Metab. (Lond.)*, **10**, No. 1, 7 (2013).
11. R. P. Miller, R. K. Tadagavadi, G. Ramesh, and W. B. Reeves, *Toxins (Basel)*, **2**, No. 11, 2490-2518 (2010).
12. N. Pabla, and Z. Dong, *Kidney Int*., **73**, No. 9, 994-1007 (2008).
13. A. P. Singh, A. Junemann, A. Muthuraman, *et al*., *Pharmacol. Rep*., **64**, No. 1, 31-44 (2012).
14. X. Yao, K. Panichpisal, N. Kurtzman, and K. Nugent, *Am. J. Med. Sci*., **334**, No. 2, 115-124 (2007).
15. I. I. Zamorskii, and T. S. Shchudrova, *Biophysics*, **59**, No. 5, 834-836 (2014).