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Nephroprotective Effect of EDL Peptide at Acute Injury of Kidneys of Different Genesis

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EDL peptide produced a nephroprotective effect on experimental models gentamycin-induced nephropathy and ischemia/reperfusion kidney injury in rats. The nephroprotective effect of EDL peptide manifested in prevention of oliguria and retention azotemia, a decrease in proteinuria and sodium excretion, prevention of critical decrease in activities of antioxidant enzymes, suppression of LPO, and normalization of energy supply to kidneys cells. Our findings confirm the prospects of further studies of the nephroprotective properties of peptide EDL in various pathologies of the kidneys.

Key Words: *acute kidney damage injury; EDL peptide; nephroprotection*

Over the last 25 years, the incidence of acute kidney injury increased by 20-fold [12] and constitutes 13-20% of all hospitalizations [8]. At the same time, 38-76% cases are accompanied by the development of ischemic or toxic acute tubular necrosis often caused by potentially nephrotoxic drugs [10]. This necessitates the search for substances that have a nephroprotective effect.

Peptide EDL characterized by hepatoprotective and nephroprotective properties under physiological conditions and during various pathologies was synthesized at the St. Petersburg Institute of Bioregulation and Gerontology. This peptide is non-toxic and causes no allergic reactions [11]. Peptide EDL stimulates the growth of organotypic cultures of the kidney and liver, regulates the expression of intercellular matrix remodeling marker MMP-14, and increases the vascular and tubular permeability of cells [2,6]. It activates cell growth, reduces the expression of aging markers of p16, p21, and p53, and increases the expression of SIRT-6 protein. Molecular modeling showed that peptide EDL forms energetically favorable complexes with $d(ATATATATAT)_2$ the sequence in the DNA small groove that may indicate its involvement into regulation of the expression of genes encoding aging proteins and functional activity of kidney cells [7]. The nephroprotective effect of EDL peptide in experimental cisplatin-induced and rhabdomyolytic acute injury to the kidneys was determined [3,4].

The goal of this article was to study the nephroprotective effect of EDL peptide on the models of toxic gentamycin-induced nephropathy and ischemia/reperfusion injury of kidneys in rats.

MATERIALS AND METHODS

Two series of experiments were carried out. We used random-bred albino rats weighing 150-200 g (n=42) that were kept under standard conditions of the vivarium with constant temperature and humidity, free access to water and mixed fodder. All studies were

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conducted in accordance with European Union Directive 2010/63/EU "On the protection of animals used for scientific purposes".

In series I, the rats were randomized into 3 groups (7 animals per group): controls (group 1); animals with gentamicin-induced nephropathy induced by administration of 4% gentamicin sulfate (Galichfarm) in a single dose of 80 mg/kg over 6 days (group 2) [13,14]; and animals with gentamicin-induced nephropathy receiving 3 μg/kg EDL peptide (intraperitoneally, 40 min after each gentamicin injection). Nephroprotective activity of the peptide in the specified dose was demonstrated in previous experiments [3,4]. The animals were sacrificed in 24 h after the last gentamicin dose and specimens for analysis were taken.

In series II, the rats were also divided into 3 groups (7 animals per group): sham operated animals (group 1), modeling of ischemia/reperfusion injury (group 2), and administration of EDL peptide in a dose of 3 μg/kg for 3 days prior to ischemia/reperfusion modeling. Ischemia was modeled under aseptic conditions under general anesthesia (sodium etaminal, 40 mg/kg) by clipping of each renal pedicle for 60 min followed by 24-h reperfusion [8,9], after that urine was collected for 2 h at conditions of induced aqueous diuresis (enteral intragastric administration of drinking water heated to 37°C in a volume of 5% of body weight). The animals were sacrificed by decapitation under ether anesthesia.

Renal function was assessed by diuresis, plasma creatinine (P_{Cr}), creatinine clearance (Cl_{Cr}), urinary protein excretion (E_{Pr}), fractionated excretion (FE_{Na}) and reabsorption (R_{Na}) of sodium ions [5]. Creatinine concentration in the plasma was measured by Popper's method modified by Merzon and in the urine by the method of Folin. Protein content in the urine was measured by the sulfosalicylic method. Sodium concentration in the blood plasma and urine was assayed by flame photometry [3]. Parameters of the renal function were standardized by recalculating absolute values per body weight unit or volume of glomerular filtrate. For evaluation of the state of the prooxidant-antioxidant balance in the kidney tissues, MDA content was measured in the reaction with TBA, catalase activity by the reaction with ammonium molybdate, and glutathione peroxidase (GPx) activity by the amount of reduced glutathione. To assess the state of energy metabolism, succinate dehydrogenase (SDH) activity in the kidney tissue was assayed by the intensity of potassium ferricyanide reduction [1].

The results were processed statistically using SPSS Statistics 17.0 software. The data distribution was determined using the Kolmogorov—Smirnov test. The differences between the samples were estimated using the Student's *t* test (in case of normal distribution) or nonparametric Mann—Whitney *U* test (when the distribution did not fit the normal law). The correlation between the variables was analyzed using Spearman's correlation coefficient (*r*). The minimum significance level was $p<0.05$.

RESULTS

Direct glomerular toxicity and accumulation of gentamicin in the epithelial cells of the renal tubules lead to destabilization of cell membranes, generation of free radicals, activation of apoptosis, massive proteolysis, energy imbalance, and inflammation that cause tubular necrosis and renal dysfunction [13].

In series I, administration of gentamicin to animals for 6 days produced a pronounced nephrotoxic effect that manifested in excretory dysfunction and oliguria (diuresis decreased by 54%), retention azotemia (P_{Cr} increased by 3.3 times and Cl_{Cr} decreased by 74%), and pronounced proteinuria (E_{Pr} increased by 2.3 times in comparison with the control). The injury to the proximal tubules of the nephron led to the simultaneous inhibition of transport systems that was manifested by an increase in FE_{Na} to 4.55% (by 9.7 times) against the background of reduced R_{Na} (by 5%; Table 1).

The administration of EDL peptide attenuated the nephrotoxic effect of gentamicin, which was seen from diuresis increase by 72%, prevention of retention azotemia (2.9-fold increase in Cl_{Cr}), and E_{Pr} decrease by 43% in comparison with untreated animals. The protective effect of the peptide on cells of the proximal tubules manifested in limitation of sodium loss with the urine, which was confirmed by FE_{Na} preserved at the control level and 3% increase in R_{Na} in comparison with the group of untreated animals. Considerably improvement of all studied parameters suggests that the peptide limits both the glomerular and tubular components of gentamicin nephrotoxicity and is confirmed by a significant direct correlation between R_{Na} and Cl_{Cr} (r=0.96), R_{Na} and E_{Pr} (r=0.77).

Oxidative stress is one of the main mechanisms of gentamicin-induced nephropathy; therefore, the study of the antioxidant properties of a potential nephroprotector in this pathology is necessary for comprehensive evaluation of the protective effect. Gentamycin induced significant activation of peroxidation processes in the kidney tissue manifested in an increase in MDA content by 93% against the background of suppressed activity of antioxidant defense enzymes (GPx by 2.8 times and catalase by 2.2 times). Administration of EDL peptide to a certain extent compensated these disorders: MDA in kidney tissue decreased by 41%, catalase and GPx activities increased by 74 and 65%, respectively, in comparison with untreated animals (Table 1).

TABLE 1. Parameters of Functional State of the Kidneys, Prooxidant-Antioxidant Balance, and SDH Activity in the Kidneys of Rat Treated with EDL Peptide under Conditions of Gentamicin-Induced Nephropathy (*M±m*)

Parameter	Control	Gentamicin-induced nephropathy+EDL	Gentamicin-induced nephropathy
Functional state of the kidneys			
Diuresis, ml	4.83±0.13	2.21±0.50**	3.80±0.62^{+}
Creatinine plasma, μmol/liter	55.63±5.98	182.73±15.08***	68.27±2.31^{+++}
Creatinine clearance, μl/min	56.49±6.34	14.88±3.61*	43.03±7.63^{++}
Protein excretion, mg/2 h	0.088±0.015	0.20±0.03**	0.116±0.03^{++}
Fractional excretion of sodium ions, %	0.47±0.08	4.55±0.60***	0.87±0.18^{+++}
Sodium reabsorption, %	99.07±0.22	94.03±1.31**	97.09±0.64^{+}
Prooxidant-antioxidant balance and SDH activity			
GPx activity, nmol/(min×mg protein)	149.34±2.4	53.59±2.50***	88.51±11.80^{++}
Catalase activity, μmol H_2O_2/(min×mg protein)	7.85±0.08	3.65±0.17**	6.34±0.07^{++}
MDA content, μmol/g protein	19.08±1.69	36.85±1.56***	21.68±1.93^{++}
SDH activity, nmol succinate/(min×mg protein)	13.35±0.50	3.34±0.86***	12.89±0.45^{+++}

Note. *p<0.05, **p<0.01, *p<0.001 in comparison with the control; ^{+}p<0.05, ^{++}p<0.01, ^{+++}p<0.001 in comparison with the parameter in gentamicin-induced nephropathy.

EDL peptide also produced a pronounced effect of the energy metabolism in the renal tissue: gentamycin reduced SDH activity by 75%, while administration of the peptide against the background of gentamicin treatment maintained enzyme activity at the control level. The effect of EDL peptide on the main pathogenetic pathways of gentamicin-induced nephropathy was confirmed by significant correlation between SDH and catalase activities (r=0.76), SDH activity and R_{Na} (r=0.89), SDH activity and Cl_{Cr} (r=0.89), GPx activity and R_{Na} (r=0.75), and FE_{Na} and MDA content in kidneys tissue (r=0.63).

In series II, 60-min ischemia followed by 24-h reperfusion induced acute vascular and tubular damage

TABLE 2. Parameters of the Functional State of the Kidneys, Prooxidant-Antioxidant Balance, and SDH Activity in the Renal Tissue in Rats Treated with EDL Peptide under Conditions of Ischemia/Reperfusion of the Kidneys (*M±m*)

Parameter	Sham-operated animals	Ischemia/ reperfusion	Ischemia/ reperfusion+EDL
Functional state of the kidneys			
Diuresis, ml	4.12±0.10	2.11±0.14**	3.97±0.34^{++}
Creatinine plasma, μmol/liter	65.24±6.08	138.57±9.60**	78.39±3.58^{+}
Creatinine clearance, μl/min	66.40±8.05	17.85±1.57**	40.26±4.64^{++}
Protein excretion, mg/2 h	0.016±0.001	0.082±0.05**	0.031±0.003^{++}
Fractional excretion of sodium ions, %	0.37±0.06	2.16±1.26**	0.55±0.23^{++}
Sodium reabsorption, %	98.89±0.31	96.80±0.41**	97.88±0.39^{+}
Prooxidant-antioxidant balance and SDH activity			
GPx activity, nmol/(min×mg protein)	128.76±3.76	63.75±2.86***	101.08±2.91^{++}
Catalase activity, μmol H_2O_2/(min×mg protein)	6.91±0.13	4.85±0.31**	7.31±0.14^{++}
MDA content, μmol/g protein	23.66±1.22	34.87±1.05**	26.34±0.58^{++}
SDH activity, nmol succinate/(min×mg protein)	12.82±0.18	2.71±0.23***	14.57±0.81^{+++}

Note. **p<0.01, ***p<0.001 in comparison with the sham-operated animals; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 in comparison with ischemia/ reperfusion.

accompanied by a decrease of diuresis by 49%, 2-fold increase of creatinemia against the background of Cl_{Cr} decrease by 73%, and 2.8-fold enhanced protein excretion in comparison with those in sham-operated animals. Inhibition of tubular reabsorption led to sodium loss, which was seen from 5.8-fold increased FE_{Na} (Table 2).

Administration of EDL peptide prevented the development of pronounced renal dysfunction in animals. Diuresis and P_{Cr} were at the control level, which attested to preserved excretory function of the kidneys and is related to a significant increase in Cl_{Cr} by 2.3 times in comparison with untreated animals. The protective effect of the peptide reduced the intensity of proteinuria: E_{Pr} decreased by 62% in comparison with the untreated group. Administration of EDL peptide led to a compensatory increase in sodium reabsorption, which in turn led to a decrease of FE_{Na} to a control level.

The protective effect of EDL peptide is determined by modulation of the main pathogenetic mechanisms of acute kidney injury: oxidative stress and energy deficiency [7]. It is known that ischemia/reperfusion is accompanied by considerable activation of free-radical processes, which is confirmed by an increase in MDA content in the renal tissue (by 47%) and inhibition of catalase and GPx (by 30 and 50%, respectively). In turn, the decrease in SDH activity by 79% attests to significant disturbances in the energy supply to the nephron under conditions of ischemic damage (Table 2).

In the group of animals treated with EDL peptide, GPx and catalase activities increased by 59 and 51%, respectively, which prevented the oxidative stress; MDA content decreased by 25% in comparison with the ischemia/reperfusion group. Administration of the peptide was followed by a 5.4-fold increase in SDH activity, *i.e.* the peptide maintains cell functioning under conditions of energy deficit. Analysis of correlations between the studied parameters in the group of animals treated with EDL peptide revealed correlations of SDH activity with MDA content (r=-0.67), FE_{Na} (r=-0.7), and Cl_{cr} (r=0.62), a correlation between catalase activity and FE_{Na} (r=-0.86), and correlations of MDA content with Cl_{cr} (r=-0.6) and GPx activity (r=-0.82).

Thus, we demonstrated nephroprotective activity of EDL peptide in nephropathy of both toxic origin and caused by ischemia/reperfusion of kidneys. Comparative evaluation of peptide influence on the course of acute renal pathology of various origins attested to its cytoprotective effect on the cells of renal tubules, which was confirmed by normalization of parameters of sodium ion transport leading to a significant increase in glomerular filtration rate by the tubular-glomerular feedback mechanism. In turn, the recovery of the filtration function of the kidneys prevents the development of retention azotemia and oliguria. It was found that preventive administration of the peptide in ischemia/reperfusion of the kidneys more markedly reduced the severity of proteinuria than in toxic nephropathy. Under conditions of gentamicin-induced nephropathy, the peptide produced more potent antioxidant effect than in ischemic damage, which was seen from more pronounced decrease in MDA content in the kidneys tissue and increase in catalase activity under the influence of the peptide.

Thus, EDL peptide produces a protective effect on the renal tissue in models of toxic gentamycin-induced and ischemia/reperfusion injury, as evidenced by recovery of the excretory function of the kidneys, improvement of energy supply to nephrons, suppression of free-radical processes, preserved activity of antioxidant defense enzymes, and the existence of correlations between the parameters.

REFERENCES

1. Aryutyunyan AV, Dubinina EE, Zybina NN. Methods for Evaluation of Free Radical Oxidation and Antioxidant System. Guidelines. St. Petersburg, 2000. Russian.
2. Bobkova IN, Kozlovskaya LV, Li OA. Matrix metalloproteinases in the pathogenesis of acute and chronic kidney diseases. Nefrologiya Dializ. 2008;10(2):105-111. Russian.
3. Zamorskii II, Shchudrova TS. Main mechanisms of rhabdomyolysis-caused kidney injury and their correction by organospecific peptides. Biophysics. 2014;59(5):834-836.
4. Zamorskii II, Shchudrova TS, Lin'kova NS, Nichik TE, Khavinson VKh. Peptides restore functional state of the kidneys during cisplatin-induced acute renal failure. Bull. Exp. Biol. Med. 2015;159(6):736-739.
5. Kamyshnikov VS. Manual on Clinical and Biochemical Tests and Laboratory Diagnostics. Moscow, 2009. Russian.
6. Khavinson VKh, Lin'kova NS, Polyakova VO, Durnova AO, Nichik TE, Kvetnoi IM Peptides regulate expression of signaling molecules in kidney cell cultures during in vitro aging. Bull. Exp. Biol. Med. 2014;157(2):261-264.
7. Khavinson VKh, Soloviev AYu, Tarnovskaya SI, Lin'kova NS. The mechanism of short peptides biological activity: cell-penetration and epigenetic regulation of gene expression. Uspekhi Sovremen. Biol. 2013;133(3):310-316. Russian.
8. Acute kidney injury: Prevention, detection and management of acute kidney injury up to the point of renal replacement therapy. NICE Clinical Guidelines. Vol. 169. London, 2013 Aug.
9. El Sabbahy M, Vaidya VS. Ischemic kidney injury and mechanisms of tissue repair. Wiley Interdiscip. Rev. Syst. Biol. Med. 2011;3(5):606-618.
10. Gill N, Nally JV Jr, Fatica RA. Renal failure secondary to acute tubular necrosis: epidemiology, diagnosis and management. Chest. 2005;128(4):2847-2863.
11. Khavinson VKh, Grigoriev EI, Malinin VV, Ryzhak GA. Peptide stimulating liver tissue regeneration, pharmacological substance based thereon and method of its application. Eurasia Patent. EA 010156. June 30, 2008.

12. Murugan R, Kellum JA. Acute kidney injury: what's the prognosis?. Nat. Rev. Nephrol. 2011;7(4):209-217.
13. Singh AP, Junemann A, Muthuraman A, Jaggi AS, Singh N, Grover K, Dhawan R. Animal models of acute renal failure. Pharmacol. Rep. 2012;64(1):31-44.
14. Quiros Y, Vicente-Vicente L, Morales AI, Lopez-Novoa JM, Lopez-Hernandez FJ. An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicol. Sci. 2011;119(2):245-256.