Shchudrova T. S., Zamorskii I. I., Korotun O. P. Evaluation of a renoprotective potential of organospecific peptides under the conditions of acute kidney injury of different etiology. Journal of Education, Health and Sport. 2018;8(1):221-227. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1188278
http://ojs.ukw.edu.pl/index.php/johs/article/view/5329

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26.01.2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 23.01.2018. Revised: 26.01.2018. Accepted: 31.01.2018.

"Pathological Physiology of Kidneys & Water Salt Homeostasis"

February, 15-16, Odessa
Materials of conference

EVALUATION OF A RENOPROTECTIVE POTENTIAL OF ORGANOSPECIFIC PEPTIDES UNDER THE CONDITIONS OF ACUTE KIDNEY INJURY OF DIFFERENT ETIOLOGY

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Abstract

Acute kidney injury (AKI) is a clinical syndrome which incidence increases dramatically over the past years in both developing and developed countries. It is considered as an independent risk factor for mortality in critically ill patients as well as complicates the course of a significant number of diseases. Consequently, there is a great need for new effective renoprotective options. Our research was conducted to evaluate the renoprotective potential of organospecific peptides (peptide kidney complex, synthetic oligopeptides AED, EDL and AEDG) in comparison with antioxidant drug mexidol (emoxypine succinate) and pineal hormone melatonin. An integral criterion of renoprotective activity was a survival rate of animals under the conditions of AKI of different genesis: induced by ethylene glycol, cisplatin, gentamicin, rhabdomyolysis and ischemia-reperfusion. All of the studied peptides decreased mortality of animals, while the most significant protective effect was shown by

peptide EDL, confirmed by 33.3% survival rate on the 5th day of ethylene glycol intoxication development and 100% survival on the other models of AKI. The least effective was peptide AED, as it prevented mortality only in animals with cisplatin-induced AKI. Obtained results show the renoprotective potential of organospecific peptides and give the background for their further study.

Key words: peptides, acute kidney injury, survival rate, renoprotection

In spite of substantial insight into the causes and pathogenesis of acute kidney injury (AKI) its incidence has increased over the past decades as has the frequency of dialysis-requiring AKI [1, 9, 14]. Taking into account its short and long-term outcomes such as development of chronic kidney disease as well as its aggravating influence on cardiovascular morbidity and mortality (15-60%), a search for the new preventive and therapeutic options is an important and topical scientific issue [4, 8, 9, 12]. In addition to pre-renal causes of renal ischemia, in more than 20% of cases AKI is caused by toxic agents, including tubular injury and acute tubular necrosis (40-70%) induced by nephrotoxic effects of drugs [11, 12]. Most often emergency situations with life-threatening kidney injury result from the influence of toxic factor on the whole organism. Incidence of AKI among the ICU patients ranges from 20% to 50%, while 10% of requiring hemodialysis cases is induced by toxins [1, 9].

One of the widespread causes of toxic kidney injury is intoxication with ethylene glycol, which damaging effect often results in irreversible kidney damage and death of patient [5]. An effective antineoplastic drug cisplatin causes severe kidney damage in 20-30% of patients [6, 10]. It is also well-known nephrotoxicity of aminoglycosides, which remain the first line antibiotics in treatment of gram-negative bacterial infections, but lately their use is restricted due to side effects [3, 10]. Rhabdomyolysis which is quite often induced by drugs and toxins leads to development of AKI in 10-40% of cases [2, 7]. Ischemia-reperfusion is a leading cause of pre-renal kidney failure [1, 8, 12].

Mentioned above facts justify use of animal models of AKI induced by ethylene glycol, cisplatin, gentamicin, rhabdomyolysis as well as ischemia-reperfusion injury for the evaluation of the possible renoprotective potential of new substances [13].

Organospecific peptides were synthetized in St. Petersburg Institute of Bioregulation and Gerontology (Russia) as a result of a study of polypeptide fractions of kidneys (peptide kidney complex (PKC), AED and EDL) and pineal gland (AEDG). They show stimulatory effect on the growth of kidney cell cultures and tissue explantants, regulating processes of cell

proliferation and apoptosis. Influence of peptides on the functional state of kidneys as well as their renoprotective effects were confirmed in our experiments [16, 17, 18].

A goal of research was to study the nephroprotective potential of peptides in conditions of toxic and ischemic AKI. Melatonin and mexidol were used as a reference drugs due to their antioxidant and nephroprotective properties confirmed by numerous studies [6, 15]. Survival rate of animals was taken as an integral criterion of renoprotective effect.

Materials and methods

Research was carried out on non-linear white rats aged 4-6 months and white mice aged 2-3 months. Animals were kept under the standard vivarium conditions with a constant temperature and humidity, free access to water and food. All studies were conducted in accordance with European Union Directive 2010/63/EU «On the protection of animals used for scientific purposes». Organospecific peptides EDL and AED at a dose of 3 μg/kg, AEDG – 7 μg/kg, PKC – 300 μg/kg, melatonin (Sigma-Aldrich, USA) at a dose of 4 mg/kg, and mexidol («Farmasoft», Russia) at a dose of 100 mg/kg were administered intraperitoneally [16-18]. Animals were randomized into groups (6-8 animals per group). Gentamicin-induced AKI was caused by administration of 4% gentamicin sulfate solution («Galychfarm», Ukraine) at a dose of 80 mg/kg over 6 days. Peptides were administered 40 min after each gentamicin injection. Rhabdomyolytic AKI was induced by a single intramuscular injection of 50% glycerol at 8 mL/kg, peptides and mexidol were administered during 7 days prior to glycerol. Cisplatin-induced AKI was modeled by a single intraperitoneal injection of cisplatin (EBEWE Pharma, Austria) at a dose of 6 mg/kg, peptides and melatonin were injected for 4 days before and 3 days after cisplatin administration. Ethylene glycol intoxication was caused by a single subcutaneous injection of solution to mice at a dose of 10 mg/kg. Ischemia was modeled in aseptic conditions under general anesthesia (sodium ethaminal, 40 mg/kg) by clipping of each renal pedicle for 60 min followed by 24-h reperfusion. Peptides and mexidol were injected for 3 days prior to ischemia/reperfusion modeling [13].

Statistical analysis of the data was performed using SPSS Statistics 17.0 software. Alternative differences were analyzed using Fisher's φ-criterion. The minimum significance level was $p<0.05$.

Results and discussion

Survival rate of mice in group of animals with ethylene glycol intoxication was 16.7% in 12 h, and 0% – 24 h after administration of toxic agent (table 1). Survival rate in group of

mice which were previously injected with PKC was increased for 1 day (33.3%) comparing to ethylene glycol AKI group, 3 days later all animals were dead. Similar results were noticed for AEDG peptide, which influence resulted in an increase of survival up to 33.3% during 1 day after ethylene glycol administration. The least potent protective effect in conditions of ethylene glycol AKI was shown by peptide AED: survival of mice 12 h after toxin administration was 33.3% ($p>0.05$), and all animals died during the next 2 days. Better results were obtained in group of mice which were treated with peptide EDL: 12 h after exposure survival was 66.7%, 2 days later – 50%, on the 5th day – 33.3%, which exceeded effect of other peptides and didn't conceded the protective effect of reference drug mexidol.

Table 1

Survival of rats in conditions of acute kidney injury and administration of organospecific peptides

Group of animals	AKI model	AKI + PKC (300 µg/kg)	AKI + EDL (3 µg/kg)	AKI + AED (3 µg/kg)	AKI + AEDG (7 µg/kg)	AKI + mexidol (100 mg/kg)	AKI + melatonin (4 mg/kg)
Ethylene glycol AKI (%, n=6)							
12 h	16,7	66,7*	66,7*	33,3	50	83,3*	n/s
1st day	0	33,3*	50*	16,7	33,3*	66,7*	n/s
2nd day	0	16,7	50*	0	16,7	50*	n/s
3rd day	0	0	33,3*#	0	0	50*	n/s
5th day	0	0	33,3*#	0	0	33,3*	n/s
Rhabdomyolysis-induced AKI (%, n=8)							
1st day	62,5	75	100*	75	87,5	100*	n/s
Cisplatin-induced AKI (%, n=7)							
4th day	85,7	100	100	100	100	n/s	100
Gentamicin-induced AKI (%, n=7)							
7th day	71,4	100*	100*	85,7	100*	n/s	n/s
Ischemia-reperfusion AKI (%, n=8)							
1st day	62,5	87,5	100*	75	87,5	100*	n/s

Note. * $p<0.05$ – in comparison with AKI, # $p<0.05$ – in comparison with other peptides, n/s – wasn't studied.

In AKI due to rhabdomyolysis a mortality rate was 37.5%. A tendency to decrease in mortality was established in groups of AEDG and EDL peptides, with 100% survival of animals treated with EDL, which equaled the effect of reference drug mexidol.

Modeling of cisplatin-induced AKI resulted in death of 14.3% of rats in group of untreated animals, while use of organospecific peptides and melatonin prevented lethal damage of kidneys.

Development of gentamicin-induced AKI was accompanied with a reduction in animal survival to 71.4%, though administration of PKC, peptides AEDG and EDL fully prevented death of animals.

A decrease in survival to 62.5% was observed in rats with ischemia-reperfusion AKI. Among the studied substances only EDL and mexidol completely prevented death of animals, while PKC and peptide AEDG showed a tendency to decrease in rats' mortality.

Among the studied peptides the most significant protective effect was shown by peptide EDL, confirmed by 33.3% survival rate on the 5^{th} day of ethylene glycol intoxication development and 100% survival on the other models of AKI. Under the influence of peptide AEDG there was a tendency to improvement of survival rate and prolongation of life duration in ethylene glycol intoxication. Use of PKC was less effective in rhabdomyolytic AKI, although on the other models its protective potential was fully compatible with the effect of peptide AEDG. Use of AED peptide prevented death of animals and provided 100% survival rate only in cisplatin-induced AKI, while on the other models lethality didn't differ from the group of untreated AKI.

Obtained results suggest that use of peptides in conditions of AKI reduces risk of lethality, giving opportunity and additional time for the appropriate therapy of intoxication or injury. Pathogenesis of AKI of any origin includes some common mechanisms such as direct or secondary damage of proximal tubular cells, obstruction of distal tubules with casts, development of acidosis, oxidative stress and inflammation, depletion of ATP content and energy deficiency, activation of apoptosis, renal vasoconstriction and ischemia. This results in failure of renal mechanisms of autoregulation, further progression of tissue damage with development of acute tubular necrosis. Hence, regulatory influence of organospecific peptides on the processes of cellular proliferation and apoptosis as well as their cytoprotective properties result in an ability to act on the main pathogenetic mechanisms of AKI.

Research results show the protective potential of peptides confirmed by an increase in kidney resistance to action of damaging factors, inhibition of pathology progression and

prevention the irreversible changes in nephrons. It opens new perspectives for the further study of organospecific peptides as a new direction in renoprotection.

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