December 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.3 • 236-243

RENOPROTECTIVE EFFICACY OF PINEAL PEPTIDE AND MELATONIN IN DRUG-INDUCED KIDNEY INJURY

Shchudrova Tetiana*, Zamorskii Igor, Kopchuk Tamara, Drachuk Vira, Korotun Olena, Dykal Mariana, Palibroda Nadiia, Lomakina Yuliia

Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" Chernivtsi, Ukraine

*tshchudrova@gmail.com

Abstract

Acute renal injury (AKI) is a common pathology affecting every fifth hospitalized patient and 40-60% of patients in the intensive care unit. Drugs are proved to cause or exacerbate 20-30% of AKI episodes, while among elderly patients the frequency of the drug-induced kidney damage reaches 66% of cases. The study was conducted to assess the renoprotective effect of melatonin and pineal peptide AEDG on the drug-induced acute kidney injury. Both peptide AEDL and melatonin demonstrated a renoprotective effect under the conditions of drug-induced kidney damage on experimental models of gentamicin, acetaminophen, and cisplatin-induced acute kidney injury in rats. The renoprotective effect manifested in the prevention of oliguria, retention azotemia, hypokalemia, a decrease in proteinuria and sodium excretion, proving the ability of pineal factors to influence the key pathophysiologic mechanisms of drug-induced nephrotoxicity and minimize damage to tubular cells. The obtained data justify a perspective for further studying and possibilities of using melatonin and peptide AEDG to prevent drug-induced nephrotoxicity.

Keywords: *drug-induced kidney injury, nephrotoxicity, melatonin, renoprotection*

Introduction

Acute renal injury (AKI) is a common pathology affecting every fifth hospitalized patient and 40-60% of patients in the intensive care unit [1, 2, 3]. The main risk factors for the AKI include preexisting kidney disease, diabetes mellitus, cardiovascular pathology, and acute dehydration. The development of AKI is accompanied by negative medical and economic consequences: higher expenditure of resource, prolonged hospitalization, and increased rate of in-hospital mortality or disability after discharge, a progression of chronic kidney disease, development of cardiovascular complications, and a higher risk of long-term mortality [4].

Medicines are proved to cause or exacerbate 20-30% of AKI episodes, while among elderly patients the frequency of the drug-induced kidney damage reaches 66% of cases [5, 6, 7]. The incidence of nephrotoxicity constantly increases due to the availability and uncontrolled use of over-the-counter drugs, unreasonable administration of drugs at high doses, and various drug-drug interactions. The common mechanisms of the drug-induced nephrotoxicity include deterioration of the renal perfusion with changes in glomerular hemodynamics, direct cytotoxicity, development of inflammation, casts formation and tubular obstruction, as well as rhabdomyolysis and thrombotic microangiopathy, while one drug can induce several types of damage [8, 9]. Therefore, nephrotoxicity may manifest in the development of acute tubular necrosis, glomerular or tubulointerstitial damage, as well as obstructive nephropathy.

The most commonly associated with nephrotoxicity are antimicrobials for systemic use, angiotensin-converting enzyme (ACE) inhibitors, diuretics, anticancer, anti-inflammatory and radiocontrast drugs [1, 3, 6, 8, 9]. The risk of aminoglycoside-induced AKI reaches 25% of cases, and increases with a long course of therapy in elderly patients, in the presence of kidney disease, diabetes mellitus, sepsis, hypovolemia, and the use of other nephrotoxic drugs. However, despite the risk and the lack of effective methods for the prevention of toxicity, aminoglycosides remain first-line drugs in the treatment of gram-negative bacterial infections [3, 7, 9, 10].

Acetaminophen (paracetamol) is one of the most widely used analgesic and antipyretic, however, an acute acetaminophen intoxication is associated with the hepatotoxicity and fulminant liver damage in 3-26% of cases. It is also known that an acetaminophen overdose leads to the development of AKI in 2-10% of cases, and in some patients, the degree of damage to the kidneys does not correlate with the degree of liver injury [11]. It should be noted, that N-acetylcysteine, which is used as an antidote in paracetamol poisoning, prevents the development of liver failure, but does not affect the incidence and course of AKI [9].

Cisplatin is among the most effective antineoplastic agents. Its main adverse effect is kidney injury occurring in 20% of patients. Strategies for preventing cisplatin nephrotoxicity include co-administration of hypertonic saline or sodium thiosulfate, however, none of these methods has shown significant clinical results, which justifies the search for effective nephroprotectors [9, 12, 13].

The pineal hormone melatonin is the main regulator of biorhythms with cytoprotective, antioxidant, immunomodulatory properties, as well as the ability to potentiate the action of antineoplastic agents (including cisplatin) [14, 15, 16]. At therapeutic doses, melatonin is not associated with the significant toxic effects, which contributes to its active study in order to expand the spectrum of clinical use, including in nephrology [17, 18, 19].

Results of our experimental studies and literature data demonstrate the renoprotective effect of the synthetic peptide AEDG (alanyl-glutamyl-aspartyl-glycine) in conditions of AKI along with its ability to stimulate the production of endogenous melatonin in the absence of toxic effects [20-24]. This gives the background to estimate its potential and possibility of use as a renoprotective agent in patients with a high risk of drug-induced kidney injury.

Based on the fact that the use of potentially nephrotoxic drugs is inevitable in many clinical situations, an understanding of pathogenic mechanisms, early detection and reduction of

nephrotoxicity is an approach to avoid the development of renal failure [25].

The aim of the research was to study the renoprotective effect of melatonin and pineal peptide AEDG on the animal models of gentamicin-, cisplatin-, and acetaminophen-induced acute kidney injury.

Methods

Three series of experiments were conducted on 90 nonlinear mature white rats weighing 150-200 g, maintained in the vivarium conditions at constant temperature and humidity, free access to water and food (full value fodder for the laboratory animals). Animals of each series were randomly divided into four groups (n=7). Group I – control; group II – AKI; group III – administration of melatonin (Sigma-Aldrich, USA) at a dose of 5 mg/kg, and group IV – administration of peptide AEDG (epitalon, Institute of Bioregulation and Gerontology, St. Petersburg, Russia) at a dose of 7 μg/kg against the background of AKI development. Doses of drugs were determined in accordance with the literature and the results of our previous research [21-24].

Cisplatin-induced AKI was caused by a single intraperitoneal injection of cisplatin (EBEWE Pharma, Austria) at a dose of 6 mg/kg 72 hours before the animals were withdrawn from the experiment [26]. Melatonin and peptide AEDG were administered 4 days prior and 3 days after the administration of cisplatin. Gentamicin nephropathy was induced by daily administration of 4% gentamicin sulfate solution (Galychpharm JSC, Ukraine) at a dose of 80 mg/kg for 6 days. Melatonin and the AEDG peptide were injected 1 hour after each injection of gentamicin. Acetaminophen-induced AKI was caused by a single intraperitoneal administration of acetaminophen (paracetamol, Health, Ukraine) at a dose of 750 mg/kg [26]. Melatonin and the AEDG peptide were administered 1 h after every paracetamol injection. Animals were withdrawn from the experiment 24 h after the last injection, while blood, urine and kidneys were sampled for biochemical and histopathological assessments. All studies were carried out in accordance with the criteria outlined in the European Union Directive 2010/63/EU "On the protection of animals used for scientific purposes" (2010).

Kidney function was evaluated by diuresis, plasma creatinine level, creatinine clearance, urine protein excretion, fractional excretion and reabsorption of sodium, and plasma potassium level. Plasma and urine creatinine levels were determined using the Jaffe reaction; urine protein content – using the sulfosalicylic acid precipitation test; sodium and potassium levels – using an electronic flame photometry method [27].

Statistical processing of the obtained data was performed using the SPSS Statistics 17.0 software. All data are represented as a mean ± standard error of the mean (M±m). Estimation of the differences between the samples was conducted using a parametric Student's t-test and a nonparametric Mann-Whitney U test. The minimum significance level was $p<0.05$.

Results

In the first series of experiments, the nephrotoxic effect of gentamicin caused the development of an oliguric form of AKI. In rats with gentamicin-induced AKI, there was a significant decrease in diuresis and creatinine clearance compared with the control group, which led to an increase in the plasma creatinine level with the development of retention azotemia (see Table 1). Disturbances in the tubular transport due to the gentamicin toxic effects resulted in an increased sodium and potassium loss and development of hypokalemia, as indicated by a significant decrease in plasma potassium level.

In the second series of experiments, a single administration of an overdose of acetaminophen to rats resulted in an excessive accumulation of the drug in the proximal tubular cells and affected sodium transport, as shown by a decrease in the relative reabsorption and, accordingly, an increase in fractional sodium excretion (see Table 2). This effect is associated with translocation and dysfunction of Na^+-K^+-ATPase, which ensures effective sodium reabsorption under normal conditions. As a result, an increase in the sodium concentration in the tubular fluid leads to activation of tubuloglomerular feedback and a decrease in GFR. In animals from the group II (acetaminophen-induced AKI), a 2-fold decrease in creatinine

clearance, reduced urine output, an increase in azotemia, and significant proteinuria compared with the control indicate on the severe damage to renal tubular cells.

In the third series of experiments (see Table 3), in animals from the group II treated with cisplatin a primary deterioration of the functional tubular activity resulted in the oliguric AKI, which was accompanied by an increase in azotemia, proteinuria, a significant sodium and potassium loss, and development of the characteristic cisplatin-associated hypokalemia.

Discussion

Nephrotoxicity is the main dose-dependent side effect of aminoglycoside therapy and is often accompanied by the development of acute tubular necrosis with clinical manifestation on the 5-7th day of therapy. Gentamicin is freely filtered through the renal glomeruli, and then it reabsorbs and accumulates in the proximal tubular cells, where its concentration exceeds the serum one by 100 times. Direct cytotoxicity leads to lysosomal phospholipidosis, functional changes in membrane transporters followed by the deterioration of the electrolyte transport in the tubules, which is accompanied by activation of peroxidation, inflammation, proteolysis, apoptosis, and energy imbalance with the development of tubular degeneration or necrosis and loss of kidney function [6, 9].

Co-administration of the experimental drugs caused a significant limitation of the gentamicin cytotoxicity. Both melatonin and peptide AEDG increased glomerular filtration rate (GFR), prevented oliguria and retention azotemia, while in rats treated with peptide AEDG, the plasma creatinine level even remained normal. Both drugs significantly reduced the degree of proteinuria, which indicates a cytoprotective effect and preservation of the reabsorption function of tubular cells confirmed by a decrease in the fractional excretion of sodium (to the control level in rats from the group IV), as well as the prevention of hypokalemia.

Data from various experimental studies show a renoprotective potential of the antioxidant and anti-inflammatory agents in animals with the gentamicin nephropathy. It has been established that the intensification of the protein and lipid peroxidation and the overproduction of reactive oxygen species is the most important pathogenetic mechanism of this nephropathy, leading to the membrane and DNA damage and morphofunctional disorders [3, 9, 10]. It can be suggested, that established in our experiment renoprotective effect of pineal factors is due to their influence on these pathogenetic mechanisms [19, 28].

The administration of acetaminophen at toxic doses leads to the excessive formation of the highly reactive metabolite N-acetyl-p-benzoquinone imine, which is normally inactivated by binding to glutathione. When glutathione stores in the hepatocytes are depleted, NAPQI binds to sulfhydryl groups of cellular proteins, which leads to mitochondrial dysfunction, the development of oxidative stress, impairment of energy metabolism, induction of apoptosis, fragmentation of nuclear DNA, and ultimately necrotic cell death with the subsequent liver and kidney malfunction [9, 11].

In animals that received peptide AEDG or melatonin treatment, renal dysfunction was less pronounced: creatinine clearance and urine output were significantly higher than in the pathology group; proteinuria and sodium excretion – significantly lower; plasma creatinine level, sodium reabsorption, and plasma potassium were within normal limits. The obtained results show the efficacy of melatonin and peptide AEDG in the prevention of mitochondrial dysfunction and its consequences at the initial stage of the acetaminophen-induced AKI development.

Cisplatin has an alike ability to accumulate in the proximal tubular cells causing damage to the mitochondria, impaired functioning of the respiratory chain and aerobic metabolism, as well as generation of reactive oxygen species, development of energy deficiency and progression of pathological changes, with a secondary injury of the glomeruli and distal tubules. Due to the similarity of the pathogenetic mechanisms, cisplatin-induced AKI is likewise characterized by a primary disturbance of tubular reabsorption due to inhibition of the membrane transport systems of the epithelial tubular cells, which leads to an increase in ion loss

followed by a decline of the renal blood flow, GFR and urine output [6, 9, 12].

The experimental data presented in Table 3 indicate a limitation of the degree of damage and impairment of kidney function in rats from the groups III and IV and show the effectiveness of the peptide AEDG and melatonin use in order to minimize the nephrotoxic effect of cisplatin and the prevent toxic AKI.

The results of the experimental research show the renoprotective effect of the pineal hormone melatonin (5 mg/kg) and the pineal peptide AEDG (7 μg/kg) under the conditions of drug-induced kidney injury. The established efficacy in preventing toxic nephropathies of various genesis suggests the universal protective effect of pineal factors due to their pleiotropic effects and the ability to influence key pathogenetic mechanisms of AKI. An important advantage of these drugs is the minimal risk of side effects, including nephrotoxicity, even after prolonged use at high doses. The obtained data substantiate further studies of the effectiveness and possibilities of using melatonin and peptide AEDG to prevent or minimize drug-induced nephrotoxicity.

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Table 1. Effect of melatonin (5 mg/kg) and peptide AEDG (7 μg/kg) on kidney function of rats with gentamicin-induced AKI

Index	*Control*	*Gentamicin-induced AKI*	*Gentamicin + Melatonin*	*Gentamicin + peptide AEDG*
Diuresis, ml/2 h	4.65±0.19	2.56±0.13[1]	3.54±0.09[2]	4.05±0.37[2]
Plasma creatinine, μmol/L	59.67±3.92	146.66±4.27[1]	103.00±4.57[2]	82.43±3.71[2,3]
Creatinine clearance, ml/min	54.79±7.85	19.14±1.58[1]	30.39±2.43[2]	44.37±4.08[2]
Urine protein excretion, mg/100 μL	0.021±0.003	0.156±0.020[1]	0.083±0.009[2]	0.053±0.005[2]
Fractional sodium excretion, %	0.60±0.07	2.60±0.50[1]	1.53±0.11[2]	0.96±0.16[2,3]
Sodium reabsorption, %	97.49±0.17	96.02±0.39[1]	96.19±0.24	97.08±0.50
Plasma potassium, mmol/L	5.39±0.27	3.36±0.26[1]	5.04±0.13[2]	5.61±0.33[2]

[1] *p<0.05 compared to control;* [2] *p<0.05 compared to gentamicin-induced AKI;* [3] *p<0.05 compared to melatonin*

Table 2. Effect of melatonin (5 mg/kg) and peptide AEDG (7 μg/kg) on kidney function of rats with acetaminophen-induced AKI

Index	*Control*	*Acetaminophen-induced AKI*	*Acetaminophen + Melatonin*	*Acetaminophen + peptide AEDG*
Diuresis, ml/2 h	5.52±0.19	3.40±0.15[1]	4.43±0.10[2]	4.82±0.19[2]
Plasma creatinine, μmol/L	67.47±4.74	115.81±6.30[1]	82.94±5.38[2]	79.40±4.79[2]
Creatinine clearance, ml/min	47.97±3.90	24.40±1.89[1]	37.65±2.30[2]	38.83±3.15[2]
Urine protein excretion, mg/100 μL	0.004±0.001	0.046±0.006[1]	0.011±0.001[2]	0.014±0.003[2]
Fractional sodium excretion, %	1.05±0.14	3.51±0.25[1]	1.51±0.18[2]	1.29±0.10[2]
Sodium reabsorption, %	96.81±0.24	93.88±0.37[1]	95.88±0.32[2]	96.63±0.33[2]
Plasma potassium, mmol/L	5.75±0.33	5.14±0.15	5.57±0.25	5.39±0.29

[1] *p<0.05 compared to control;* [2] *p<0.05 compared to acetaminophen-induced AKI*

Table 2. Effect of melatonin (5 mg/kg) and peptide AEDG (7 μg/kg) on kidney function of rats with cisplatin-induced AKI

Index	*Control*	*Cisplatin-induced AKI*	*Cisplatin + Melatonin*	*Cisplatin + peptide AEDG*
Diuresis, ml/2 h	4.34±0.11	1.48±0.09[1]	2.64±0.10[2]	2.93±0.18[2]
Plasma creatinine, μmol/L	55.97±2.43	124.74±4.17[1]	96.25±3.90[2]	104.34±6.47[2]
Creatinine clearance, ml/min	70.52±2.43	13.84±1.08[1]	36.06±2.33[2]	26.16±3.52[2]
Urine protein excretion, mg/100 μL	0.012±0.001	0.083±0.012[1]	0.033±0.00[2]	0.048±0.008[2]
Fractional sodium excretion, %	0.46±0.04	2.82±0.33[1]	1.09±0.08[2]	1.47±0.23[2]
Sodium reabsorption, %	98.19±0.22	95.79±0.27[1]	96.67±0.15	96.13±0.48
Plasma potassium, mmol/L	5.71±0.21	4.36±0.27[1]	5.86±0.36[2]	5.11±0.27[2]

[1] *p<0.05 compared to control;* [2] *p<0.05 compared to cisplatin-induced AKI*