RENAL EFFECTS OF THE SULFUR-CONTAINING AMINOACID DERIVATIVES (ADEMETIONINE, TAURIN AND GLUTATHIONE) IN CONDITIONALLY HEALTHY ANIMALS

Derivatives of sulfur-containing aminoacids (ademetionin, taurine and glutathione) selected for the experimental studies are used in various fields of practical medicine due to their multifaceted pleiotropic effects. The study of their renal effects in the conditions of the physiological norm makes it possible to expand and supplement their pharmacological characteristics, and forms the basis for further study of the nephroprotective potential in renal pathology.

**Materials and methods.** The experiments were performed on mature non-linear white rats weighing 130-180 g. Animals were divided into 4 groups (n = 7): group I – intact control, group II – animals which were given ademetionine ("Gepral", "Abbott Spa", Italy) in the dose of 20 mg/kg, group III – animals received taurine ("Sigma-Aldrich", USA) in the dose of 100 mg/kg, group IV – animals received glutathione ("TAD 600", "Biomedica Foscama", Italy) in the dose of 30 mg/kg. All drugs were injected intramuscularly for 7 days.

**Results.** The study of the renal effects of the sulfur-containing amino acid derivatives (SAD) under research in the daily administration for 7 days to conditionally healthy animals showed that these drugs had a weak diuretic effect, which was probably due to a decreased tubular reabsorption of water without significant changes in the glomerular filtration rate. Besides, the use of ademetionine resulted in a slight reduction of azotemia. There was not any significant change in the acid regulatory kidney function under the effect of SAD. Administration of ademetionine, taurine and glutathione resulted in a significant decrease in proteinuria, which was probably caused by the effect on the processes of protein re-absorption. The effect on the ion regulatory kidney function upon the course of SAD administration was characterized by an increase in urinary sodium excretion against the background of decreased relative sodium reabsorption, which was accompanied by intensification of distal transport of sodium ions due to the activation of the tubular-tubular balance.

**Conclusions.** According to the data obtained the 7-day administration of ademetionine, taurine and glutathione to conditionally healthy animals moderately affects the processes of glomerular filtration and tubular transport in nephrons, which results in a slight increase in diuresis along with preservation of the renal mechanisms of autoregulation and the absence of histopathological changes in the kidneys.

**Key words:** renal effects; ademetionine; taurine; glutathione
В. М. Драчук, И. И. Заморский, Т. С. Щудрова, А. М. Горошко
Высшее государственное учебное заведение Украины «Буковинский государственный медицинский университет»

Ренальные эффекты производных серосодержащих аминокислот (адеметионина, таурина и глутатиона) при применении у условно здоровых животных

Избранные для экспериментального исследования производные серосодержащих аминокислот (адеметионина, таурина и глутатиона) благодаря своим многогранным плейотропным эффектам применяются в различных областях практической медицины. Исследование их ренальных эффектов в условиях физиологической нормы дает возможность расширить и дополнить их фармакологическую характеристику, создает основу для дальнейшего изучения нефропротекторного потенциала при патологии почек.

Цель
Исследование влияния производных серосодержащих аминокислот (адеметионина, таурина и глутатиона) на морфофункциональное состояние почек крыс после курсового введения препаратов.


Результаты. Изучение ренальных эффектов исследуемых производных серосодержащих аминокислот (ПСА) при 7-дневном введении условно здоровым животным позволило установить, что исследуемые препараты оказывают слабое диуретическое действие, которое вероятно обусловлено уменьшением канальцевой реабсорбции воды при отсутствии достоверных изменений скорости клубочковой фильтрации. При этом из исследуемых препаратов только адеметионин оказывает незначительное гипотоническое действие. При исследовании состояния кислоторегулирующей функции почек не выявлено значительных изменений. В группах животных, которым вводили адеметионин, таурин и глутатион, наблюдается достоверное уменьшение проницаемости, обусловленное влиянием на процессы реабсорбции белка. При курсовом введении ПСА влияние на ионорегулирующую функцию почек характеризовалось ростом экскреции на фоне уменьшения относительной реабсорбции ионов натрия, что сопровождалось усилием дистального транспорта ионов натрия за счет активации канальцево-канальцевого баланса. По данным морфологического исследования патологических гистоструктурных изменений ткани почек под влиянием препаратов не выявлено, что свидетельствует об отсутствии нефротоксических свойств исследуемых ПСА.

Выводы. На основании проведенного эксперимента можно сделать вывод, что при 7-дневном введении условно здоровым животным исследуемые препараты адеметионин, таурин и глутатион незначительно усиливают диурез и оказывают слабое влияние на процессы клубочковой фильтрации и канальцевого транспорта в нефронах при сохранении внутрипочечных механизмов авторегуляции и отсутствии патологических изменений гистоструктуры почек.

Ключевые слова: ренальный эффект; адеметионин; таурин; глутатион.

To date, in spite of the achievements of the pharmaceutical industry, the problem of prevention and treatment of acute kidney injury (AKI), which prevalence reaches 31 % and the mortality rate exceeds 80 % of patients, remains an urgent and unsolved issue of modern nephrology [1]. According to the literature data the key link in the pathogenesis of the renal pathology is development of the oxidative stress, which is characterized by the prooxidant-antioxidant imbalance [2] and involves a shift of the redox equilibrium towards free radical oxidation with formation of lipid and protein peroxides [3]. Consequently, the promising direction is the use of nephroprotectors with the aim of strengthening the antioxidant defense, and inducing the membrane protective and cytoprotective mechanisms. It is known that sulfur-containing amino acids presented at the pharmaceutical market of Ukraine by such drugs as ademetionine, taurine and glutathione possess these properties.

Ademetionine is an active sulfur-containing metabolite of methionine, a natural antioxidant and antidepressant. Endogenous ademetionine is involved in transmethylation reactions, promoting the synthesis of phosphatidylcholine in the cell membrane and providing the membrane protective action [4]. In the transsulfuration reactions it acts as a precursor of sulfur compounds (cysteine, taurine and glutathione); in transmethylation reactions it participates in the synthesis of catecholamines (dopami-
As a medication, ademetionine belongs to the group of hepatoprotectors, but due to its pleiotropic properties it possesses the cytoprotective, anti-inflammatory, analgesic, anti-depressant, neuroprotective and antiepileptic effect [7].

Glutathione acts as the most powerful antioxidant due to the reactivity of SH-group [8]. The system of glutathione plays an important role in maintaining the thiol-disulphide balance in tissues, which is essential for implementation of such processes as the functioning of membrane structures and cytoskeleton, cell division [9-11]. As a medicine it is represented at the market in a form of sodium salt of recovered glutathione, and is used to prevent nephrotoxicity and hepatotoxicity of cisplatin, as well as in acute and chronic hepatitis, liver cirrhosis, etc. [12].

Taurine is a sulfur-containing amino acid that participates in a wide range of physiological processes, such as conjugation of bile acids, which prevents cholestasis, and stabilization of photoreceptor cells of the eye retina. Taurine is one of the main cell regulators due to its effect on the transport processes of sodium and chloride ions, it acts as a neuromodulator in the central nervous system, has potential anti-inflammatory, antioxidant, and detoxifying properties, possesses antiarrhythmic and inotropic effects [13-17]. Taurine is produced in a form of one-component eye drops and biologically active additives [18].

Considering the pleiotropic effects of sulfur-containing amino acid derivatives (SAD) – ademetionine, taurine and glutathione allowing their use in different pathologies their renal effects in physiological conditions are of special interest. It may complement the characteristics of the drugs studied and provide the background for further study of their nephroprotective potential in different kidney pathologies.

The aim of the study was to determine the effects of SAD on the morphofunctional state of the rat kidneys in the daily administration for 7 days.

Materials and methods

The experiments were performed on mature non-linear white rats weighing 130-180 g, maintained in the vivarium with the constant temperature and humidity, and with free access to water and food. Animals were divided into 4 groups (n = 7): group I – intact control, group II – animals which were given ademetionine ("Geptral", "Abbott SpA", Italy) in the dose of 20 mg/kg, group III – animals received taurine ("Sigma-Aldrich", USA) in the dose of 100 mg/kg, group IV – animals received glutathione ("TAD 600", "Biomedica Foscama", Italy) in the dose of 30 mg/kg. All drugs were injected intramuscularly for 7 days. The studies were conducted in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" [19].

The functional status of the rat kidneys was assessed on day 8. Urine was collected for 2 h under the conditions of induced water load (enteral administration of drinking water heated to 37°C in the amount of 5 % of the body weight using an intragastric tube) by the indices of diuresis, glomerular filtration rate (GFR), urine protein level and excretion, urine pH, excretion of titrated acids and ammonium ions, reabsorption and urine excretion of sodium and potassium ions [20]. The morphological examination of kidney histological sections was performed by staining the samples with hematoxylin and eosin. The preparations were assessed using light microscopy and photographed. Documentation of the pathological processes was performed by the computer morphometry of objects using "VideoTest – Razmer 5.0" computer software (Russian Federation). The statistical analysis of the data was performed using SPSS 17.0 software. All data are presented as a mean ± standard error of the mean (M±m). A character of distribution within the group was determined using Kolmogorov-Smirnov test. Estimation of the differences between the samples was conducted using parametric Student’s t-test (for normal distribution) and nonparametric Mann-Whitney U test (when the assumptions of the t-test were not met). The critical level of significance was accepted at p<0.05.

Results and discussion

The 7-day administration of ademetionine resulted in some changes in the functional state of kidneys (Table). In the experimental group II an increase in diuresis by 15.9 % along with a significant decrease in water reabsorption by 1.1 % was observed, while there were no changes in GFR compared to the control group.

The hypoazotemic effect of ademetionine was also found, it was manifested by a decrease in the plasma creatinine level by 19.9 % compared to the control group. The dosage regimen of the drug was effective in reducing proteinuria: the urine protein level decreased by 1.3 times, urine protein excretion was accordingly decreased compared to the control group. Ademetionine had no significant effect on the acid-regulatory kidney function: there was a tendency towards an increase in the urine pH and preservation of titrated acids and excretion of ammonium ions at the control level. Ademetionine had no significant effect on the acid-regulatory kidney function: there was a significant increase in the excretion of sodium ions with urine against the background of a decrease in relative reabsorption of sodium ions. The correlation between GFR and absolute reabsorption of sodium ions (r = 0.96),
The effect of sulfur-containing aminoacid derivatives (ademetionine, taurine and glutathione) on the functional state of kidneys of conditionally healthy rats (M±m, n=7)

<table>
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<tr>
<th>Index</th>
<th>Intact control</th>
<th>Ademetionine (20 mg/kg)</th>
<th>Taurine (100 mg/kg)</th>
<th>Glutathione (30 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis, ml/2 h×100 g</td>
<td>3.78±0.19</td>
<td>4.38±0.15*</td>
<td>4.89±0.13**</td>
<td>4.60±0.09**</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>59.00±2.23</td>
<td>49.22±1.83*</td>
<td>53.61±2.26</td>
<td>61.53±1.90</td>
</tr>
<tr>
<td>Glomerular filtration rate, μl/min×100 g</td>
<td>536.6±26.03</td>
<td>545.54±36.31</td>
<td>593.35±49.61</td>
<td>573.79±23.90</td>
</tr>
<tr>
<td>Reabsorption of water, %</td>
<td>99.29±0.02</td>
<td>98.22±0.02**</td>
<td>97.15±0.06**</td>
<td>97.19±0.04**</td>
</tr>
<tr>
<td>Urine protein, g/l</td>
<td>0.022±0.001</td>
<td>0.017±0.001**</td>
<td>0.011±0.001**</td>
<td>0.015±0.001**</td>
</tr>
<tr>
<td>Urine protein excretion, mg/100 μl</td>
<td>0.083±0.002</td>
<td>0.072±0.001**</td>
<td>0.053±0.001**</td>
<td>0.068±0.001**</td>
</tr>
<tr>
<td>Urine pH</td>
<td>7.07±0.11</td>
<td>7.35±0.07</td>
<td>7.24±0.10</td>
<td>7.16±0.12</td>
</tr>
<tr>
<td>Titrated acids excretion, μmol/2 h</td>
<td>27.30±1.35</td>
<td>26.68±1.63</td>
<td>24.76±1.36</td>
<td>22.44±1.90</td>
</tr>
<tr>
<td>Ammonium ions excretion, μmol/2 h</td>
<td>33.41±2.19</td>
<td>37.56±2.63</td>
<td>31.33±2.04</td>
<td>31.20±1.83</td>
</tr>
<tr>
<td>Plasma Na⁺, mmol/l</td>
<td>135.36±4.45</td>
<td>142.50±4.53</td>
<td>136.07±2.37</td>
<td>133.57±4.75</td>
</tr>
<tr>
<td>Excretion of Na⁺, μmol/2 h</td>
<td>1.93±0.09</td>
<td>3.76±0.21**</td>
<td>3.28±0.17**</td>
<td>3.62±0.50**</td>
</tr>
<tr>
<td>Absolute Na⁺reabsorption, μmol/min</td>
<td>80.83±5.19</td>
<td>93.42±7.19</td>
<td>97.02±8.81</td>
<td>91.73±4.49</td>
</tr>
<tr>
<td>Relative Na⁺reabsorption, %</td>
<td>98.37±0.10</td>
<td>96.86±0.18**</td>
<td>97.26±0.14**</td>
<td>96.99±0.42**</td>
</tr>
<tr>
<td>Proximal transport of Na⁺, mmol/min</td>
<td>9.23±0.60</td>
<td>10.62±0.84</td>
<td>10.98±1.04</td>
<td>10.40±0.53</td>
</tr>
<tr>
<td>Distal transport of Na⁺, μmol/min</td>
<td>470.98±27.70</td>
<td>593.38±33.61*</td>
<td>662.46±24.37**</td>
<td>609.99±24.68**</td>
</tr>
<tr>
<td>Plasma K⁺, mmol/l</td>
<td>5.18±0.25</td>
<td>5.71±0.51</td>
<td>5.21±0.28</td>
<td>5.36±0.44</td>
</tr>
<tr>
<td>Excretion of K⁺, μmol/2 h</td>
<td>23.15±1.42</td>
<td>23.26±0.96</td>
<td>18.25±1.87</td>
<td>21.25±1.42</td>
</tr>
</tbody>
</table>

Note. Significant differences compared to the intact control group – * (p<0.05), ** (p<0.01).

and between GFR and proximal sodium transport (r = 0.86) confirms the maintenance of the glomerular-tubular balance. In addition, under the effect of the drug there was an increase in distal sodium transport by 1.3 times along with a tendency towards a reduction of proximal sodium transport, indicating the activation of tubular-tubular balance (r = -0.83). At the same time, ademetionine did not affect the transport of potassium ions.

The study of the renal effects of taurine revealed a slight diuretic effect, which was confirmed by a significant increase in diuresis by 29.4 % compared to the control group (Table). The use of taurine did not affect the plasma creatinine level. There was a tendency towards an increase in GFR along with a decrease in water reabsorption compared to the intact animals. At the same time, administration of taurine resulted in the most pronounced effect on protein transport among the drugs studied: there was a 2-fold decrease in the urine protein level and reduction of protein excretion by 1.6 times compared to the control group. There were no significant changes in the acid-regulatory kidney function after taurine administration for 7 days. In the ion-regulatory kidney function a significant increase (by 1.7 times) in sodium excretion was observed due to an increase in diuresis and decrease in relative sodium reabsorption. The glomerular-tubular balance was maintained when administrating taurine, and it was confirmed by the correlation between GFR and absolute reabsorption of sodium ions (r = 1), GFR and proximal transport of sodium ions (r = 0.96). The tubular-tubular balance was characterized by an increase in distal transport of sodium ions (1.4 times) in the absence of changes in proximal sodium transport and characterized by a reverse correlation between proximal and distal transport of sodium ions (r = -0.89). Like ademetionine, taurine did not affect the processes of potassium transport.

When studying the effect of glutathione on the excretory function of the rat kidney an increase in diuresis by 21.7 % was revealed due to a decrease in tubular reabsorption of water in the absence of significant changes in GFR and the plasma creatinine level (Table). Glutathione also showed a pronounced effect on the tubular transport of protein: the urine protein level was decreased by 1.5 times, protein excretion – by 1.2 times compared to the control animals. Under the effect of glutathione a decrease in titrated acid excretion by 17.8 % was observed without significant changes in the urine pH compared to the intact animals. Although the index of absolute sodium reabsorption did not differ significantly from the control level, the relative sodium reabsorption decreased by 1.4 %, which led to a 1.3-fold increase in the distal transport of sodium by the mechanism of the tubular-tubular balance activation. The glomerular-tubular balance was confirmed by the correlation between GFR and the absolute sodium reabsorption (r = 0.86), as well as between GFR and proximal transport of sodium (r = 0.96). The functioning of the tubular-tubular ba-
Balance was expressed in the negative correlation between proximal and distal reabsorption of sodium ($r = -0.93$). There were no changes in the plasma potassium level and its excretion.

The histological examination of the kidneys of rats received ademetionine (Fig. 1A) revealed signs of reversible hydropic swelling in 5% of proximal tubular epithelial cells mostly in the juxtaglomerular zone within the physiological range. These changes may be due to the effect of ademetionine on the processes of water, protein, sodium and potassium transport in proximal tubules.

In group of rats which were taken taurine during the morphological examination there were no histopathological changes in the renal tissue, confirming the absence of nephrotoxic effects (Fig. 1B). According to the results of the morphological study the 7-day administration of glutathione did not cause any changes in the morphological organization of the renal tissue (Fig. 1C): glomeruli were of normal structure and size, the interstitium and epithelium of the proximal and distal tubules were without pathological changes, lumens of tubules were free.

The study of the renal effects of SAD in the daily administration for 7 days to conditionally healthy animals revealed a weak diuretic effect, which was probably due to a decreased tubular reabsorption of water without significant changes in GFR. Thus, the use of ademetionine resulted in a slight reduction of azotemia. The use of SAD did not cause any significant effect on the acid regulatory kidney function. Administration of ademetionine, taurine and glutathione resulted in a significant reduction of proteinuria, which was probably caused by the effect on the processes of protein reabsorption. The effect on the ion regulatory kidney function during the course of SAD administration was characterized by an increase in urinary sodium excretion against the background of decreased relative sodium reabsorption, which was accompanied by intensification of distal transport of sodium ions due to the activation of the tubular-tubular balance. The morphological examination did not reveal any histopathological changes in the renal tissue, confirming the absence of nephrotoxicity of the SAD studied.

CONCLUSIONS

According to the results of the experiment conducted daily administration of the sulfur-containing amino acids derivatives (ademetionine, taurine and glutathione) for 7 days to conditionally healthy animals causes a weak diuretic effect with little impact on the processes of glomerular filtration and tubular transport in nephrons, accompanied with maintenance of the renal mechanisms of autoregulation, and the absence of histopathological changes in the kidneys.

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References


References


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Drachuk V. M., teaching assistant of the Pharmacology Department, Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (http://orcid.org/0000-0003-3208-7609). E-mail: vira.drachuck2017@gmail.com

Драчук В. М., асистент кафедри фармакології, Вищий державний медичний університет України «Буковинський державний медичний університет» (http://orcid.org/0000-0003-3208-7609). Е-mail: vira.drachuck2017@gmail.com

Zamorskii I. I., Doctor of Medicine (Dr. habil), professor of the Pharmacology Department, Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (http://orcid.org/0000-0003-0947-6729). E-mail: igorzamorskii@gmail.com

Заморський І. І., доктор медичних наук, професор, завідувач кафедри фармакології, Вищий державний медичний університет України «Буковинський державний медичний університет» (http://orcid.org/0000-0003-0947-6729). Е-mail: igorzamorskii@gmail.com

Shchudrova T. S., Candidate of Medicine (Ph.D.), associate professor of the Pharmacology Department, Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (http://orcid.org/0000-0003-4186-2013). E-mail: tshchudrova@gmail.com

Щудрова Т. С., кандидат медичних наук, доцент кафедри фармакології, Вищий державний медичний університет України «Буковинський державний медичний університет» (http://orcid.org/0000-0003-4186-2013). Е-mail: tshchudrova@gmail.com

Goroshko O. M., Candidate of Pharmacy (Ph.D.), associate professor of the Pharmacy Department, Higher State Educational Establishment of Ukraine "Bukovinian State Medical University" (http://orcid.org/0000-0002-1341-3010). E-mail: gorolesya@ukr.net

Горошко О. М., кандидат фармацевтичних наук, доцент кафедри фармації, Вищий державний медичний університет України «Буковинський державний медичний університет» (http://orcid.org/0000-0002-1341-3010). Е-mail: gorolesya@ukr.net

Address for correspondence: 2, Teatralna sq., Chernivtsi, HSEE of Ukraine "Bukovinian State Medical University", Pharmacology Department.

Адреса для листування: м. Чернівці, пл. Театральна, 2, кафедра фармакології ВДНЗ України «Буковинський державний медичний університет». Моб. тел. +380663978774. Е-mail: vira.drachuck2017@gmail.com

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