

Functional renal state of mature rats with a quick type of acetylation under conditions of cadmium chloride and sodium nitrate

T.M. Boychuk, T.I. Kmet, N.D. Filipets, L.I. Vlasyk, T.I. Hrachova

Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi; kmet.taras@bsmu.edu.ua

The experiments conducted on laboratory non-linear albino mature male rats with a quick type of acetylation examined renal function under conditions of cadmium-nitrate intoxication. A combined introduction of cadmium chloride (0.1 mg/kg intraperitoneally) and sodium nitrate (500 mg/kg intragastrically) during 14 days was found to result in 31.2 % increase of glomerular filtration rate (GFR). Due to increased GFR volume regulating, excretory kidney functions are activated that was assessed by endogenic creatinine clearance. In case of lack of hypercreatininemia the concentration of creatinine in the urine 16.5 % increased, creatinine excretion became 31.8 % higher. Concentration of sodium ions in the urine 67.8 % decreased, and excretion with urine decreased three times as much. Concentration of sodium ions in the blood plasma 6.6 % decreased. Although renal regulation of sodium ions metabolism was not disturbed: distal reabsorption did not change, proximal reabsorption 24.4 % increased. Retention of tubular mechanisms of sodium metabolism regulation and glomerular-tubular balance are indicative of adaptive possibilities of the kidneys of mature rats with a quick type of acetylation ensuring maintenance of water-electrolytic balance under conditions of subacute cadmium-nitrate effect. Further investigations of the effect of a combined action of cadmium chloride and sodium nitrate on mature rats with slow acetylation and comparative analysis of the renal function are of great importance in order to assess a prognostic value of acetylation phenotype in the development of nephropathy.

Key words: quick acetylizers; cadmium chloride; sodium nitrate; kidneys.

INTRODUCTION

An active search of the markers of individual susceptibility of the body to the effect of toxicogenic environmental factors as well as occurrence and development of different pathological conditions has been conducted recently. The priority of acetylation processes in metabolism and genetic determination of their rate forms the basis for the study of interrelations of acetylizer types with the efficacy of medicines and sickness rate [1-4]. A continuous increase of nephrological patients with naturally determined progress of nephron loss induced us to specify the role of acetylation phenotype in the formation of nephropathy. Considering age dependence of physiological

processes and reactions of metabolism, the fragment of the study was conducted only on the mature rats with a quick type of acetylation. It should be noted that cadmium chloride and sodium nitrate as simulated poisonous substances have been chosen due to the three reasons. First, both substances are the most global pollutants of the environment. Second, under real conditions their combined action occurs. Third, cadmium salts and nitrates induce nephropathy [5-9].

The objective of our study was investigation of the functional kidney state of mature rats with a quick type of acetylation under conditions of a combined effect of cadmium chloride and sodium nitrate.

METHODS

The study was conducted on laboratory non-linear albino male rats 6 months of age with the body weight of 0.16-0.18 kg, kept under standard vivarium conditions on balanced feeding. Acetylation ability of rats was determined according to certain methods [10]. Rats with a quick type of acetylation were divided into 2 groups: I – control (intact) group, II – group with simulated subacute cadmium-nitrate intoxication. To simulate the model rats were injected with cadmium chloride during 14 days intraperitoneally in the dose of 0.1 mg/kg (1/150 DL₅₀) and sodium nitrate intragastrically (through the probe) in the dose of 500 mg/kg (1/15 DL₅₀). During the whole period of the experiment the control group was injected with isotonic solution of sodium chloride (intraperitoneally) and settled tap water (intragastrically) instead of external poisonous substances. 24 hours after the last introduction all the rats were subjected to 5 % water load (tap water, intragastrically) and placed into exchange cages to collect urine. Diuresis was registered every 2 hours, blood was taken in the amount of 20 mg/kg from rats narcotized by means of 1 % of sodium ethaminal solution. The concentration of sodium ions in urine and blood plasma was determined by means of flame photometry method; creatinine in urine – by means of Folin's method, in the blood plasma – by means of Poper's method in Merson's modification [11]. Parameters of the kidney function standardized by the body mass and glomerular filtrate volume were calculated by the common formulas [12]. The findings were statistically processed by means of the program «Statgrafics», consistency of data were assessed by Student t-criterion.

RESULTS

According to the data obtained presented in the Table mature rats with a quick type of acetylation under conditions of a combined effect of cadmium chloride and sodium nitrate developed

13.4 % increase of diuresis, and a relative diuresis – by 8.8 %. Relative reabsorption of water did not change. Increased urination in case of a stable permeability for water of the kidney tubular walls was caused by the activation of glomerular processes: glomerular filtration rate (GFR) became 31.2 % as much. Concentration of creatinine in the blood plasma did not change. In case of hypercreatininemia lack creatinine in urine increased by 16.5 % due to increased secretion in the tubules. Creatinine concentration index increased by 15.6 %, excretion increased by 31.8 %. The concentration of sodium ions in the blood plasma decreased by 6.6 %, 23 % increase of the filtration fraction of sodium ions was indicative of a considerable increase of sodium loading of the nephron tubular portion. At the same time, the concentration of sodium ions in urine decreased by 67.8 %, and the indices of its secretion with urine decreased three times as much. The fraction of sodium ions increased by 23.2 % which is reabsorbed and its relative reabsorption increased, and excretory fraction decreased by 64.1 %. The clearance of sodium-free water increased respectively (by 14.7 %), that characterizes not only reabsorption of sodium ions in the tubules together with anions, but distal transport of this electrolyte as well. Renal mechanisms of maintenance of sodium ions in the body were not disturbed. Reabsorption in the distal tubules of a nephron did not change, proximal reabsorption of sodium ions increased by 24.4 %.

DISCUSSION

Under conditions of cadmium-sodium effect in mature rats with a quick type of acetylation water excretion and renal excretory functions were activated. It should be noted that cadmium ions in the body are fixed with low molecular protein metallothionein and cumulated in the kidneys. Structural-functional peculiarities including a high rate of kidney blood supply provide the effect of external toxic substance in the tubular and glomerular portions of the nephron with

Functional kidney state after introduction of cadmium chloride (0.1 mg/kg intraperitoneally) and sodium nitrate (500 mg/kg intragastrically) during 14 days to mature rats with a quick type of acetylation (M±m, n=7)

Indices	Quick acetylizers	
	Control	Cadmium chloride and sodium nitrate
Diuresis, ml/2 h	3.3±0.18	3.7±0.08 (P<0.05)
Relative diuresis, %	66.7±3.78	75.7±1.73 (P<0.05)
Urine Na ⁺ , mmol/l	2.3±0.11	0.74±0.154 (P<0.001)
Na ⁺ excretion, μmol/2 h	7.7±0.62	2.7±0.54 (P<0.01)
Urine creatinine, mmol/l	0.92±0.041	1.10±0.058 (P<0.05)
Plasma creatinine, μmol/l	69.2±0.60	69.6±0.56
Glomerular filtration rate, μl/min	369.7±23.22	485.1±26.20 (P<0.05)
Creatinine concentration index, units	13.3±0.51	15.4±0.76 (P<0.05)
Creatinine excretion, μmol/2 h	3.1±0.19	4.0±0.22 (P<0.01)
Relative H ₂ O reabsorption, %	92.4±0.29	93.3±0.38
Plasma Na ⁺ , mmol/l	136.7±1.86	127.8±0.87 (P<0.01)
Filtration fraction of Na ⁺ , μmol/min	50.3±2.67	61.9±3.36 (P<0.05)
Excretory fraction of Na ⁺ , μmol/min	0.06±0.005	0.02±0.004 (P<0.01)
Reabsorption fraction of Na ⁺ , μmol/min	50.3±2.67	61.9±3.36 (P<0.05)
Relative reabsorption of Na ⁺ , %	99.8±0.01	99.9±0.01 (P<0.001)
Clearance of H ₂ O Na ₊ , ml/2 h	3.2±0.18	3.7±0.08 (P<0.05)
Excretion of Na ⁺ , μmol/100 μl GF	2.1±0.11	0.6±0.14 (P<0.001)
Proximal reabsorption of Na ⁺ , mmol/2 h	5.5±0.30	6.9±0.40 (P<0.05)
Distal reabsorption of Na ⁺ , μmol/2 h	447.4±21.15	481.4±12.46

Notes: P – reliability in comparison with the control rats; n – number of rats in every group; GF – glomerular filtration.

injury of the endothelial and epithelial cells. Similar to other heavy metals, nephrotoxic action of cadmium salts promotes tubular dysfunction with disorders of proximal transport first of all [5]. In our investigation with small doses of exotoxins than those necessary for the induction of toxic and hypoxic nephropathy, reabsorption of sodium ions in the distal tubules did not change. Increased proximal transport of this main osmotic active electrolyte was indicative of preservation of the renal mechanisms maintaining sodium balance. The ability of

kidneys to excrete concentrated or diluted urine depends on the distal reabsorption which is influenced by many regulators of water-electrolyte metabolism. Increased diuresis and excretion of water free from sodium ions could be a result of decreased activity of vasopressin in response to hyponatremia and due to neurotoxicity of cadmium chloride [13]. Cadmium is known to be deposited in those parts of the brain where hematoencephalic barrier is easily penetrated – the pituitary gland and pineal body [14, 15].

Nephrotoxic action of cadmium salts is

characterized, as a rule, by glomerular and interstitial changes prevailing in damage of the tubular epithelium [5]. It should be noted that according to the results [16], all the patients with glomerulonephritis and interstitial nephritis residing in the region with an increased content of heavy metals in the environment were quick acetylizers. As compared to other observations where cadmium intoxication caused decrease of filtration processes [7], increased GFR in our study reflects not only protective response of the body to exotoxins but effects of sodium nitrate. Water-regulatory and excretory function of the kidneys assessed by the concentration index and excretion of endogenic creatinine increased due to increased GFR. In case of acute blood hypoxia simulated by nitrates and nitrites, GFR can be unchanged or increase due to preserved blood supply against the ground of reduced oxygen capacity of blood [5]. Moreover, nitrates and nitrites are direct products of nitrogen oxide metabolism, and they can be involved into the repeated cycle of nitrogen oxide performing the function of its depot and transport. It is not ruled out that under conditions of the experiment increased GFR is caused by the activation of NO-dependent vasodilation. At the same time, increased GFR, increased filtration fraction and tubular loading with sodium ions were associated with increased proximal reabsorption which is indicative of preserved glomerular-tubular balance.

It should be noted that in the previous studies we have investigated changes of biochemical indices in the blood plasma of rats of different acetylizers under similar conditions of cadmium-nitrate intoxication. A quick type of acetylation was found to be a biological marker of susceptibility to the effect of cadmium chloride and sodium nitrate in mature rats, and the criteria of a harmful action in them may be the following: increased concentration of methemoglobin, increased TBA-active products of lipid peroxide oxidation, increased oxidative modified proteins and level of medium molecular peptides, decreased hemoglobin level [17].

At the same time, kidneys play a leading role in endogenic protection due to stability of the renal mechanisms, involvement of the functional renal reserve and rather long maintenance of homeostasis under conditions of the body injury [18, 19]. Therefore, changes of the renal functional state on the 14th day of cadmium-sodium effect in mature male rats with a quick type of acetylation are adaptive-compensator reactions to provide water-salt balance at the expense of increased glomerular filtration and regulation of tubular reabsorption.

CONCLUSIONS

1. A combined effect of cadmium chloride in the dose of 0.1 mg/kg and sodium nitrate in the dose of 500 mg/kg during 14 days in mature male rats with a quick acetylation type due to increased glomerular filtration rate promotes activation of water excretion and excretory function of the kidneys.

2. The lack of changes of distal transport and increased proximal reabsorption of sodium ions are indicative of the retention of tubular mechanisms to regulate sodium metabolism.

3. Availability of glomerular-tubular balance – the most important physiological mechanism of water-salt metabolism regulation – is indicative of maintenance of adaptive possibilities of kidneys of mature rats with a quick acetylation type under conditions of subacute cadmium-nitrate effect.

4. Investigation of a combined effect of cadmium chloride and sodium nitrate on mature rats with a slow acetylation type with comparative analysis of the renal function to assess a prognostic value of acetylation for the development of nephropathy is promising.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of coauthors of the article.

**Т.М. Бойчук, Т.І. Кметь, Н.Д. Філіпец,
Л.І. Власик, Т.І. Грачова**

**ФУНКЦІОНАЛЬНИЙ СТАН НИРОК
СТАТЕВОЗРІЛИХ ЩУРІВ ЗІ ШВИДКИМ
ТИПОМ АЦЕТИЛЮВАННЯ ЗА УМОВ
ВПЛИВУ ХЛОРИДУ КАДМІЮ ТА НІТРАТУ
НАТРІЮ**

В експериментах на лабораторних нелінійних білих статевозрілих щурах-самцях зі швидким типом ацетилювання досліджено функції нирок за умов кадмієво-нітратної інтоксикації. Показано, що після комбінованого введення впродовж 14 діб хлориду кадмію (0,1 мг/кг внутрішньоочеревинно) і нітрату натрію (500 мг/кг внутрішньошлунково) зростає на 31,2 % швидкість клубочкової фільтрації. Завдяки цьому активується водовидільна, а також екскреторна функція нирок, яку оцінювали за кліренсом ендogenous креатиніну. За відсутності гіперкреатинемії концентрація креатиніну в сечі зростала на 16,5 %, екскреція креатиніну – на 31,8 %. Концентрація іонів натрію в сечі зменшувалась на 67,8 %, екскреція з сечею – знижувалась утричі. Концентрація іонів натрію в плазмі крові зменшувалась на 6,6 %. Ниркова регуляція обміну іонів натрію не порушувалась: дистальна реабсорбція не змінювалась, підвищення проксимальної реабсорбції становило 24,4 %. Збереженість каналцевих механізмів регуляції натрієвого обміну та клубочково-каналцевого балансу свідчать про адаптаційні можливості нирок статевозрілих щурів із швидким типом ацетилювання, які забезпечують підтримку водно-електролітної рівноваги за умов підгострого кадмієвого-нітратного впливу. Важливе практичне значення мають подальші дослідження комбінованої дії хлориду кадмію та нітрату натрію на статевозрілих щурів повільних ацетиляторів із порівняльним аналізом функцій нирок для оцінки прогностичного значення фенотипу ацетилювання у розвитку нефропатій.

Ключові слова: швидкі ацетилятори; хлорид кадмію; нітрат натрію; нирки.

*Вищий державний навчальний заклад України
«Буковинський державний медичний університет»,
Chernivtsi; e-mail: kmet.taras@bsmu.edu.ua*

**Т.Н. Бойчук, Т.И. Кметь, Н.Д. Филипец,
Л.И. Власык, Т.И. Грачева**

**ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ
ПОЧЕК ПОЛОВОЗРЕЛЫХ КРЫС
С БЫСТРЫМ ТИПОМ АЦЕТИЛИРОВАНИЯ
ПРИ ВОЗДЕЙСТВИИ ХЛОРИДА
КАДМИЯ И НИТРАТА НАТРИЯ**

В экспериментах на лабораторных нелинейных белых, половозрелых крысах-самцах с быстрым типом ацетилювания исследованы функции почек в условиях

кадмиево-нитратной интоксикации. Установлено, что при комбинированном введении в течение 14 сут хлорида кадмия (0,1 мг/кг внутрибрюшинно) и нитрата натрия (500 мг/кг внутривентриально) возрастает скорость клубочковой фильтрации, активируются водовыделительная и экскреторная функции почек, увеличивается проксимальная реабсорбция ионов натрия. Сохранение канальцевых механизмов регуляции натриевого обмена и клубочково-канальцевого баланса свидетельствуют об адаптационных возможностях почек половозрелых крыс с быстрым типом ацетилювания, которые обеспечивают поддержку водно-электролитного равновесия в условиях подострого кадмиево-нитратного воздействия. Перспективными являются исследования комбинированного действия хлорида кадмия и нитрата натрия на половозрелых крыс с медленным типом ацетилювания со сравнительным анализом функций почек для оценки прогностического значения фенотипа ацетилювания для развития нефропатии.

Ключевые слова: быстрые ацетиляторы; хлорид кадмия; нитрат натрия; почки.

REFERENCES

1. Kravchenko IE, Fazylov VCh, Zaripova RG. Metabolic enzyme systems activity evaluation in patients with streptococcal tonsillitis and ways of pharmacologic correction with ksimedon. Bull Contemp Clin Med. 2013; 6 (3): 8-12. [Russian].
2. Khalili H, Dashti-Khavidaki S, Amini M, Mahjub R, Hajiabdolbaghi M. Is there any difference between acetylator phenotypes in tuberculosis patients and healthy subjects? Eur J Clin Pharmacol. 2010; 66(3): 261-7.
3. Pons D, deVries FR, van den Elsen PJ, Heijmans BT, Quax PH, Jukema JW. Epigenetic histone 37 acetylation modifiers in vascular remodelling: new targets for therapy in cardiovascular disease. Eur Heart J. 2009; 30(3): 266-77.
4. Wang Q, Zhang Y, Yang C, Xiong H, Lin Y, Yao J, Li H, Xie L, Zhao W, Yao Y, Ning ZB, Zeng R, Xiong Y, Guan KL, Zhao S, Zhao GP. Acetylation of metabolic enzymes 31 coordinates carbon source utilization and metabolic flux. Science. 2010; 327(5968): 1004-7.
5. Boychuk TN, Gordienko VV, Rohovoy YuYe. Chronorhythms of the kidneys: the age-old aspect under the conditions of metallotoksikoza: a monograph. Chernivtsi: Medical University. 2016; 179. [Ukrainian].
6. Boychuk TN, Rohovoy YuYe, Popovich GB. Pathophysiology of hepatorenal syndrome in hemic hypoxia. Chernivtsi: Medical University. 2012; 192. [Ukrainian].
7. Gozhenko AI, Dolomatov SI, Dolatova EA, Karchauskas VY, Pychteyev DM. Function of the kidneys with cadmium nephropathy under conditions of water and salt loading. Nephrology. 2002; 6 (3): 75-8. [Russian].
8. Filipets ND, Sirman VM, Gozhenko AI. The mechanisms of ion regulation kidneys function in histohemic hypoxia and possible ways of its correction. Nephrology. 2014;

- 18(4): 57-1. [Russian].
9. Filipets ND, Gozhenko AI, Sirman VM. Effect of modulators of ion channels on renal function at the initial stage of development of histohemic hypoxia. J NAMS Ukraine. 2014; 20(4): 483-7. [Ukrainian].
 10. Pershin GN. Methods of experimental chemotherapy (practical guide) / ed. GN. Pershina: 2nd edition. Moscow: Medicine, 1971; 453-7. [Russian].
 11. Berkhin EB., Ivanov YuI. Methods of experimental study of the kidneys and water-salt metabolism. Barnaul: Altai Book, Publishing House, 1972; 199. [Russian].
 12. Ryabov SI, Natochin YuV. Functional Nephrology. SPb.: Lan, 1997; 304. [Russian].
 13. Bernard A. Confusion about Cadmium Risks: The Unrecognized Limitations of an Extrapolated Paradigm. Environm Health Perspectiv. 2016; 124(1): 1-5.
 14. Kmet TI, Vlasyk LI. Dynamics of changes of behavioral reactions in albino rats of juvenile age with a diverse types of acetylation under conditions of a subacute effect of cadmium chloride and sodium nitrate. Buk Med Herald. 2003; 7(3): 111-4. [Ukrainian].
 15. Peng Q, Bakulski KM, Nan B, Park SK. Cadmium and Alzheimer's disease mortality in U.S. adults: Updated evidence with a urinary biomarker and extended follow-up time. Environ Res. 2017; 157: 44-1.
 16. Soblirova ZnKh, Kharina EA. Is Rapid Acetylation a Predictor of Kidney and Urinary Tract Diseases. Nephrol and Dialysis. 1991; 1(1): 20-3. [Russian].
 17. Kmet TI. Hygienic meaning of combined effect of sodium nitrate and cadmium chloride with taking into consideration age peculiarity and metabolism character. The Dissertation submitted for the Candidate of Sciences (Medicine) Degreet. Kyiv, 2006; 20. [Ukrainian].
 18. Gozhenko AI, Kravchuk AV, Nikitenko OP, Moskolenko OM, Sirman VM. Functional Renal Reserve. Odessa: Fenix. 2015; 180. [Ukrainian].
 19. Gozhenko AI., Filipets ND. The functional state of kidneys after adenosine triphosphate-sensitive potassium channels activation in experimental acute hypoxia. Fiziol Zh. 2014; 60(4): 22-9. [Ukrainian].

Received 16.11.2017