UDC **616.36-008.6-092**

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PATHOGENESIS OF KIDNEY AND LIVER LESIONS UNDER CONDITIONS OF 2,4-DINITROFENOL ADMINISTRATION

Key words: *2,4-dinitrofenol, kidneys, liver, cytokines, sodium ions loss syndrome, melatonin, oxidative modified proteins.*

Abstract. *From the positions of probative medicine the work presents theoretical substantiation and a new approach to solve the scientific task concerning early pathogenesis mechanisms of pseudohepatorenal syndrome as the basis to deteriorate the course of kidney and liver failure with breaking oxidative phosphorylation under conditions of 2,4-dinitrofenol administration. The experiments were conducted on 120 albino outbred male rats with the body weight of 0,16-0,20 kg fed on low-sodium diet with tissue hypoxia modeling.*

Introduction

2,4-dinitrofenol administration is known to cause the development of acute tissue hypoxia [4] due to the break of oxidative phosphorylation resulting in renal disorders with disturbance of the main energy-dependent process – reabsorption of sodium ions and proteins in the proximal portions of the nephron, and liver lesions [1]. Cytokines, the products of protein oxidative modification, may promote the development of early mechanisms of liver and kidney lesions with the formation of syndromes of translocation and sodium ions loss with urine. The antioxidant melatonin is reasonable to be used as means of pathogenic correction.

Objectives

To specify early mechanisms of liver and kidney lesions under conditions of low sodium diet with acute tissue hypoxia caused by 2,4-dinitrofenol administration with elaboration of ways to correct pathogenesis of the lesions detected by means of melatonin use.

Materials and methods

Acute tissue hypoxia was modeled by means of single intraperitoneal injection of 0.1% 2,4-dinitrofenol solution in the dose of 3 mg/kg in the experiment conducted on 120 male albino outbred rats with the body weight of 0,16-0,20 kg [3].

Rats' resistance to acute hypoxia was estimated by the time of their position loss on the "high-altitude plateau" of acute hypobaric hypoxia and the time of general animal stay from the moment of reaching the "height" of 12000 m to the appearance of the second agonic inspiration (life time or reserve time), as well as by the time of restoration of the position from the beginning of the descending moment. There were three groups of animals divided: highly, average, and low resistant [2]. All the following study was conducted on the average resistant rats.

The portions of the liver and kidney tissues during 48 hours were fixed in 10% neutral buffered formalin solution, followed by dehydration procedure in the ascending ethanol battery and paraffin coating at the temperature 58^0C. To estimate protein oxidative modification histological sections were stained with bromphenol blue. Computed spectrometry was made by means of ColorPic computer program (Graphic Art Tools, 2004). Histochemical technique to determine the ration between the principal and acid groups of proteins was based on the measurement of intensity of red and blue spectrum colours during computer-spectral analysis of microscopic objects digital images and calculation of R/B coefficient, as the ratio between the staining intensity in the portion of red spectrum (R) and in the portion of blue one (B) [7, 10].

Functional state of the kidneys was examined by water diuresis simulated by tap water injected intragastrically through a metal probe and heated to 37^0C in the volume of 5% out of body weight. The volume of diuresis (V) was estimated in ml/2 hours ^{M} 100 g. After water load with the aim to obtain plasma the animals were decapitated under mild ether narcosis, the blood was taken into tubes with heparin. Glomerular filtration rate was estimated by endogenic creatinine clearance calculated by the formula:

$$C_{cr} = U_{cr}{}^{M} V/P_{cr,}$$

where U_{cr} and P_{cr} stand for creatinine concentration in the urine and blood plasma respectively. The concentration of sodium and potassium ions in the urine and blood plasma was estimated by photometry, protein concentration in the urine was detected by sulfosalicylic method. Proximal and distal reabsorption of sodium ions was examined

(T^pNa^+, T^dNa^+). The calculations were made by the formulae:

$$T^pNa^+ = (C_{cr} - V)^M PNa^+$$

$$T^dNa^+ = (PNa^+ - UNa^+)^M V \text{ [8]}$$

Blood cytokines were detected by immuno-enzymatic method [9]. Exogenic melatonin was injected in the single dose of 3,5 mg/kg [10].

Statistical calculation of the data obtained was made by the computer program "Statgrafics" and "Excell 7.0". All the experiments were conducted according to the European Council Convention on vertebrate animal protection used in experiments and other scientific research (dated 18.03.1986), the European Union Direction No 609 (dated 24.11.1986), the Orders of the Ministry of Public Health of Ukraine No 960 (dated 23.09.2009) and No 944 (dated 14.12.2009).

Results and Discussion

The results of the experiment demonstrated increased concentration of tumour necrosis factor-α, interleukin-1β, interleukin-6 in the blood plasma (fig.1) 2 hours after 2,4-dinitrofenol injection in the dose of 3 mg/kg under conditions of low sodium diet.

Under conditions of 2,4-dinitrofenol injection urination reduced, the concentration of potassium ions, proteins in the urine increased, GFR was inhibited (table 1). Creatinine concentration in the blood plasma and urine did not change. The transport

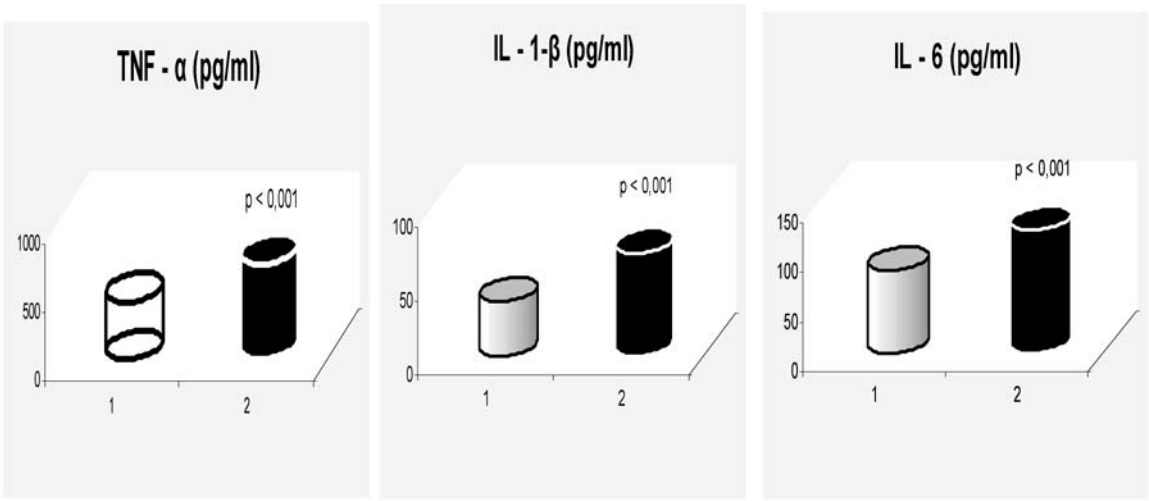

Fig.1. Concentrations of tumour necrosis factor-α, interleukin-1β, interleukin-6 in the blood plasma 2 hour after 2,4-dinitrofenol injection in the dose of 3 mg/kg under conditions of low sodium diet with water-induced diuresis in the volume of 5% out of body weight. 1 – control; 2 – 2,4 –dinitrofenol injection; p – difference probability as compared with the control

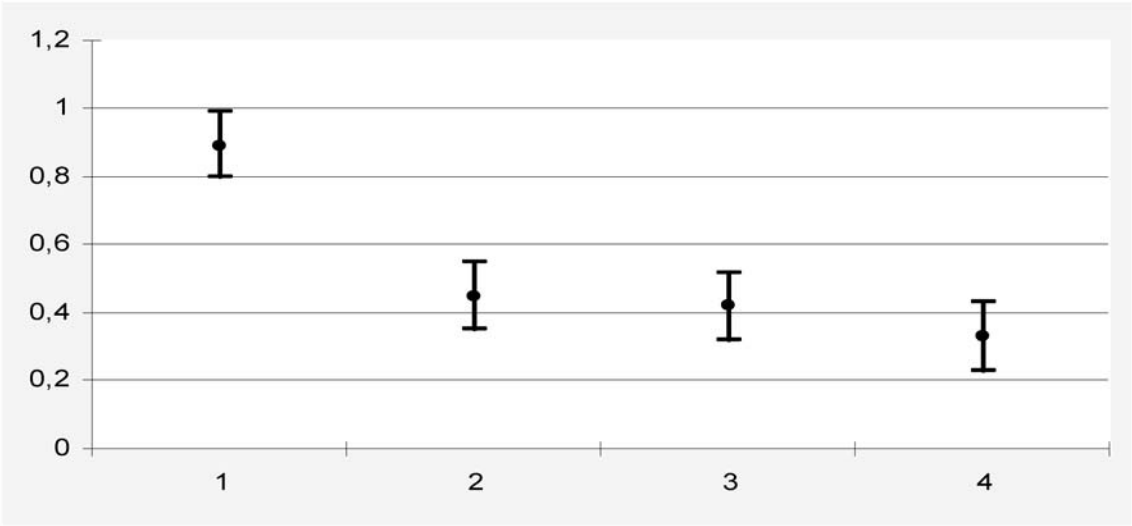

Fig. 2. Forest-diagram of the comparative characteristics of melatonin protective effect upon the epithelial cytoplasm R/B coefficient of the proximal tubules, sodium ions excretion, sodium ions clearance, concentration index of sodium ions 2 hours after 2,4-dinitrofenol injection in the dose of 3 mg/kg under conditions of low sodium diet with water-induced diuresis in the volume of 5% out of body weight. 1 – epithelial cytoplasm R/B coefficient of the proximal tubules (standard units); 2 – sodium ions excretion – (mkmol/2 h x 100g); 3 – sodium ions clearance (ml/2h x 100g); 4 – concentration index of sodium ions (standard units). The control for all the experiments is presented by a horizontal line and assumed as 1

Table 1

Indices of renal function 2 hours after 2,4-dinitrofenol injection in the dose of 3 mg/kg under conditions of low sodium diet with water-induced diuresis in the volume of 5% out of body weight (x±Sx)

Indices	Control	2, 4 – dinitrofenol (n=10)
Diuresis, ml/2 h · 100 g	4,58±0,299	3,72±0,253 p< 0,05
Concentration of potassium ions in the urine, mmol/L	15,30±1,963	26,90±4,394 p< 0,02
Creatinine concentration in the urine, mmol/L	1,46±0,086	1,41±0,058
Creatinine concentration in the blood plasma, mmol/L	53,50±3,106	47,10±2,030
Glomerular filtration, mkl/min x 100g	1096,6±136,57	919,1±42,61
Protein concentration in the urine, g/L	0,019±0,0037	0,032±0,0063
Concentration of sodium ions in the urine, mmol/L	0,37±0,053	0,76±0,061 p< 0,001
Concentration of sodium ions in the blood plasma, mmol/L	135,5±1,89	136,0±1,675
Distal reabsorption of sodium ions, mkmol/2 h x 100g	618,1±40,88	503,5±34,73 p< 0,05
Proximal reabsorption of sodium ions, mmol/2 h x 100g	17,29±2,293	14,53±0,811

Note. p – probability of differences as compared with the control; n – number of observations

Table 2

Degree of oxidative protein modification by the increase of R/B index (standard units) with histochemical analysis of the kidney and liver sections of rats poisoned by 2,4-dinitrofenol and under melatonin action (X±Sx)

Structure, where R/B index was measured	Groups of examination		
	Control, intact animals (n = 8)	2,4-dinitrofenol injection (n = 8)	2,4-dinitrofenol injection against melatonin background (n = 8)
Epithelial cytoplasm of the proximal tubules	1,08±0,005	1,39±0,009 p < 0,001	1,24±0,008 p_1< 0,001
Epithelial cytoplasm of the medullar thick ascending portions of the nephron loop	1,04±0,004	1,18±0,007 p < 0,001	1,05±0,004 p_1< 0,001
Epithelial cytoplasm of tubules of the renal papillae	1,03±0,004	1,15±0,008 p < 0,001	1,04±0,005 p_1< 0,001
Protein mass of hepatocyte cytoplasm	1,12±0,006	1,26±0,009 p < 0,001	1,19±0,008 p_1< 0,001

Note. p – probability of differences as compared with the control; p_1 – probability of differences as compared with 2,4-dinitrofenol injection; n – number of observations

of sodium ions under conditions of 2,4-dinitrofenol injection was characterized by increased concentration of sodium ions in the urine. Proximal reabsorption of sodium ions was characterized by the tendency to inhibition, and distal reabsorption of this electrolyte was reliably decreased. The concentration of sodium ions in the blood plasma was not changed.

Fig.2 presents forest-diagram of the comparative characteristics of melatonin protective effect upon the epithelial cytoplasm R/B coefficient of the proximal tubules, sodium ions excretion, sodium ions clearance, concentration index of sodium ions 2 hours after 2,4-dinitrofenol injection in the dose of 3 mg/kg under conditions of low sodium diet with water-induced diuresis in the volume of 5% out of body weight. The control for all the experiments is presented by a horizontal line and assumed as 1. As it is seen from the diagram the most protective effect under conditions of the experiment was produced by melatonin upon the concentration index of sodium ions.

2,4-dinitrofenol injection resulted in the intensification of oxidative protein modification by the increase of R/B index in the proximal tubules, medullar thick ascending portions of the nephron loop, accumulating tubules of the renal papillae, and in the protein mass of hepatocyte cytoplasm (table 2).

2,4-dinitrofenol injection caused an average twice reduction of ATP level in the renal tubules [2] at the expense of breaking oxidative phosphorylation. ATP deficiency caused disorders of the main energy-dependent process of the renal tubules – sodium ions reabsorption, leading to the development of the

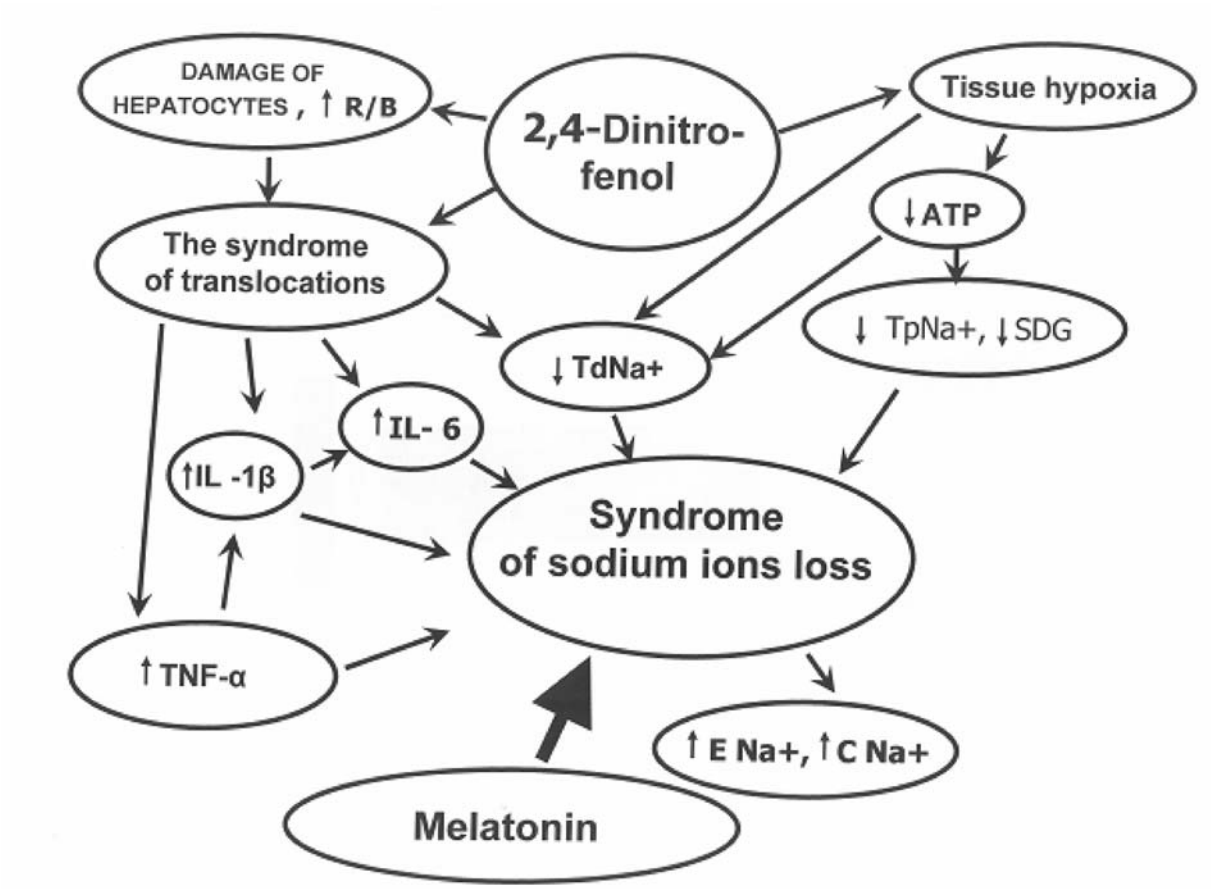

Fig.3. Generalized scheme of pseudohepatorenal syndrome pathogenesis under conditions of 2,4-dinitrofenol injection.

Note: ENa^+ - excretion of sodium ions, T^pNa^+ - proximal reabsorption of sodium ions, T^dNa^+ - distal reabsorption of sodium ions, CNa^+ - clearance of sodium ions, TNF-α – tumour necrosis factor-α, IL-1β – interleukin-1-β, IL-6 – interleukin-6, R/B – R/B coefficient of the quantitative content of protein oxidative modification products, ATP – adenosine triphosphate, ↑ - quantitative increase of the examined parameter, ↓ - quantitative decrease of the examined parameter

examined cation loss. The above mentioned facts are evidenced by the increased concentration of sodium ions in the urine. The tendency to reduce proximal reabsorption of sodium ions is stipulated by "obscured" lesions of the proximal nephron portion [5], and a reliable decrease of the examined cation distal reabsorption is caused by the fact, that transport processes in the distal tubule are more energy-dependent than in the proximal nephron portion. At the same time, the degree of loss syndrome was not considerable, as the concentration of sodium ions in the blood plasma was not changed, and not considerable activation of rennin-angiotensin-aldosterone system caused only reliable decrease of diuresis, increased concentration of potassium ions in the urine with the tendency to inhibit glomerular filtration.

Barrier lesions of the intestine and liver against a background of energy-deficiency resulted in the translocation of endotoxin from the intestinal lumen into the blood [6], which in its turn, caused increased concentration of tumour necrosis factor-α, interleukin-1-β, interleukin-6 [2, 3, 4], which in their turn, provoked additional reactions of renal tubules lesions with intensification of sodium ions loss syndrome.

On the basis of the data obtained a generalized scheme of pseudohepatorenal syndrome pathogenesis under conditions of 2,4-dinitrofenol injection can be suggested [11, 12] , reflecting a theoretical substantiation and new solution of a scientific task concerning early mechanisms of pseudohepatorenal syndrome pathogenesis as the basis to deteriorate the course of renal and liver failure with breaking oxidative phosphorylation under conditions of 2,4-dinitrofenol injection (fig.3).

Under conditions of low sodium diet 2 hours after modeling tissue hypoxia and 2,4-dinitrofenol injection increased concentration of TNF-α, IL-1β and IL-6 in the blood plasma was found, increased degree of oxidative modified proteins in the liver and kidneys of rats by R/B coefficient was detected, causing lesions of the liver and kidneys, disorders of energy metabolism with the development of the syndrome of translocation and sodium ions loss with

urine and its secretion increase. Melatonin due to its antioxidant properties demonstrated its protective effect upon the course of pseudohepatorenal syndrome under conditions of tissue hypoxia, revealing its protective influence upon the degree of oxidative modified proteins in the liver and kidneys of rats, reduced the level of R/B coefficient in the proximal, and distal portions of the nephron, accumulating tubules of the renal papillae and protein mass of hepatocyte cytoplasm as compared with the readings under 2,4-dinitrofenol intoxication.

Conclusion

From the positions of probative medicine the experimental work, conducted on outbred mature male rats, presents theoretical substantiation and a new approach to solve the scientific task concerning early pathogenesis mechanisms of pseudohepatorenal syndrome as the basis to deteriorate the course of kidney and liver failure with breaking oxidative phosphorylation under conditions of 2,4-dinitrofenol administration.

Prospects of further research

To specify the role of antiinflammatory cytokines in pathogenesis of pseudohepatorenal syndrome under conditions of 2,4-dinitrofenol injection.

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ПАТОГЕНЕЗ УШКОДЖЕННЯ НИРОК ТА ПЕЧІНКИ ЗА УМОВ УВЕДЕННЯ 2,4 - ДИНІТРОФЕНОЛУ

Ю. Є. Роговий, В. В. Белявський, Л. О. Філіпова, В. А. Дорошко, О. В. Злотар

Резюме. У дослідах на 120 білих нелінійних щурах-самцях масою 0,16- 0,20 кг при гіпонатрієвому раціоні харчування за моделювання тканинної гіпоксії з позицій доказової медицини наведено теоретичне узагальнення та нове вирішення наукової задачі щодо ранніх механізмів патогенезу псевдогепаторенального синдрому як основи погіршення перебігу ниркової та печінкової недостатності при роз'єднанні окиснення і фосфорилювання за умов уведення 2,4-динітрофенолу.

Ключові слова: 2,4-динітрофенол, нирки, печінка, цитокіни, синдром втрати іонів натрію, мелатонін, окисномодифіковані білки .

ПАТОГЕНЕЗ ПОВРЕЖДЕНИЯ ПОЧЕК И ПЕЧЕНИ ПРИ ВВЕДЕНИИ 2,4 - ДИНИТРОФЕНОЛА

Ю.Е.Роговый, В.В.Белявский, Л.О.Филипова, В.А.Дорошко, О.В.Злотарь

Резюме. В опытах на 120 белых нелинейных крысах-самцах массой 0,16-0,20 кг при гипонатриевом рационе питания в условиях моделирования тканевой гипоксии с позиций доказательной медицины представлено теоретическое обобщение и новое решение научной задачи относительно ранних механизмов патогенеза псевдогепаторенального синдрома как основы ухудшения течения почечной и печеночной недостаточности при расщепление окисления и фосфорилирования при введении 2,4-динитрофенола.

Ключевые слова: 2,4-динитрофенол, почки, печень, цитокины, синдром потери ионов натрия, мелатонин, окислительномодифицированные белки.

Clin. and experim. pathol.- 2013.- Vol.12, №3 (45).-P.146-150.

Надійшла до редакції 03.09.2013
Рецензент – проф. В.І.Швець