

Research Article



Experimental Study of Nephroprotective Properties of Sodium Poly-(2,5-Dihydroxyphenilen)-4-Thiosulfate Acid

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ABSTRACT

An article describes results of antihypoxant drug sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid effects on filtration-excretory renal function in experimental renal insufficiency. Use of sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid 90 mg/kg increases diuresis in rats, reduces endogenous creatinine level in the blood and increases its excretion in urine by strengthening glomerular filtration and normalization in tubular reabsorption. Also the studied drug positively affects renal function of nitrogen excretion in rats, increases excretion of urea, endogenous creatinine and consequently decreases their levels in animals' blood. Proved studies show the effect of the drug on the severity of proteinuria and degree of protein loss. Antihypoxant drug sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid is shown to provide complex diuretic, hypoazotemic and anti-proteinuric effects and nephroprotective action in experimental renal insufficiency and is believed to be actual for the further studying as a nonspecific nephroprotective drug.

Keywords: Antihypoxant drug, kidneys, filtration-excretory function of the kidneys, creatinine, urea, glomerular filtration, tubular reabsorption, proteinuria, experimental acute renal insufficiency, nephroprotection.

INTRODUCTION

Pathologies of kidney in the structure of WHO recent years becomes a pandemic problem. Regardless of the factors that trigger this pathology, sometimes it reaches 35.5% of the visceral pathology and cause serious consequences and disability. Especially dangerous acute renal failure, what treatment achieved only through the help of special equipment, enabling artificial kidney hemodialysis, transplant organ.

Acute renal failure (ARF) - a syndrome characterized by rapid loss of homeostatic kidney function observed in 5% of all hospitalized patients and among patients with predominant surgical and obstetric departments. Epidemiological studies in recent years, conducted in different regions of the world show that the prevalence of ARF currently stands 8% -35.5% of world population over the age of 20 years. Due to the tendency to chronic progressive drop in kidney function, ARF affects the quality and duration of patient's life.

In order to improve approaches of chronic renal failure diagnostic and its subsequent treatment proposed new term – "acute kidney damage" (acute kidney injury) and found a criteria for the classification of the severity of the syndrome RIFLE (the risk, injury (lesion), failure, loss of function, end-stage renal failure) by modification AKIN (Acute Kidney Injury Network, 2004). According to RIFLE classification system early stages of ARF is determined by any of the indicators: increase in serum creatinine level

by 1.5-1.9 times distinct to original level or decrease in glomerular filtration rate of 25% or more, a decrease in urine output to a level of less than 0.5ml/kg/hour for 6 hours¹⁻⁴.

Acute kidney damage has a wider range than the ARF, and covers all manifestations of renal disease, including minor changes of renal function, and includes sudden imbalance of fluid, electrolyte and acid-base balance by reducing the clearance of small solutes and decrease in GFR.

RIFLE based on the proposed common objectives to prevent ARF: adequate hydration and maintain renal blood flow, limitation the impact of nephrotoxins, nephroprotective therapy, and renal replacement therapy, although the authors do not provide clear recommendations for therapeutic tactics depending on the degrees of kidney damage. Among the criteria for assessing the degree of acute disorders of kidney function considering the urea content in blood plasma and urine, fractional excretion of sodium (to differentiate between prerenal and renal ARF) biomarkers - interleukin-18, molecule kidney damage-1, lipokalin associated with jelatouse of neutrophils⁵⁻⁷.

The mechanisms of kidney injury and recovery are complicated, but important for understanding the pathogenetic therapy. Whatever the cause of ischemic ARF and toxic kidney damage characterized by common mechanisms that cause acute tubular necrosis, and



specific etiologic manifestations of ARF dependent on offset to the extent of the syndrome⁸⁻¹⁰.

Primary mechanisms that cause kidney damage progression related with decompensation in a stable hypertension and/or edema, complications of the cardiovascular system, cognitive disorders, anemia, dismetabolic nephropathy.

In most cases to affect on kidney damage is almost impossible, and the only way of saving the lives of patients is renal replacement therapy (hemodialysis, peritoneal dialysis or a kidney transplant). In the absence of effective therapy approximately 50% of ARF ends with fatality¹¹⁻¹³.

Therefore, in view of the high cost of hemodialysis, peritoneal dialysis, kidney transplantation and the significant impact of these treatments on quality of life and the constant increase in the number of such patients, current treatments including research of new effective drugs with nephroprotective action. This will delay the need for renal replacement therapy, because today in Ukraine are mainly 45-52 years people (contingent of working age) who, due to disability can not fully work.

Modern pharmacological protection of kidneys mainly including the use of drugs with diuretic action, which, depending on the mechanism of diuretic impact is not always facilitate kidney function, and it may even worsen and weaken their excretory function^{1,14-16}.

General pathological processes that occur in conditions of acute renal failure (violation in blood flow, vasoconstriction, ischemia of tissues, oxidative stress) leads to search of the effective agents with antihypoxic, antioxidant properties and other metabolic compounds.

Given the above, it should be nephroprotectors with polytropic action that would have anti-hypoxic, antioxidant, membrane stabilizing, anti-inflammatory, antisensitizing, diuretic effects¹⁷⁻²⁰.

Some of that specific properties has sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid (PDT-Na), what improves tissue respiration in conditions of hypoxia, especially in organs with high metabolism, such as kidneys; reduces the occurrence of toxic lipid peroxidation products formation^{21,22}.

PDT-Na rapidly absorbed, proving efficacy in acute diseases, and has a high degree of safety. However, the drug has not been studied as nephroprotective agent and research on the background of experimental renal disease have a positive impact, preventing or slowing of its development and possible chronic flow.

Medical effectiveness of this drug in nephrology has an economic way of high-restrictive use of drugs substitution and symptomatic therapy. In turn, achieving economic efficiency will realize social needs, improving the quality of life of patients with kidney disease, increasing its durability, ability to work and be useful to society.

MATERIALS AND METHODS

Experiments performed on 24 white mongrel mature nonlinear albino rats of both sexes weighing 150-170 g. Experiments conducted in the laboratory of Department of Pharmacology and Prescription writing (Kharkiv National Medical University (KhNMU), Kharkiv, Ukraine).

All experiments were conducted according to the European convention for the protection of vertebrate animals used for experimental and other scientific purposes [Strasbourg, 1986] and according to the guidelines of the State Expert Center Ministry of Health of Ukraine (Protocol No 9 meeting of the Commission on Ethics and Bioethics KhNMU, 03.12.2014)²³⁻²⁵.

According to the study design of PDT-Na nephroprotective activity for experimental ARF used glycerol (mioglobinurical, rabdomiolitycal) model, which is one of the most comprehensively studied¹⁻⁴. It recreates crush syndrome which is caused by intramuscular injection of glycerol solution in rats. Rhabdomyolysis and intravascular hemolysis are important causes of this nephrotoxic ARF. Rhabdomyolysis leads to ARF as a result of excessive accumulation of hemoglobin in the tubules of the nephron. Myoglobin shows toxic effects and promotes units that interfere with normal fluid motion and cause the generation of reactive oxygen. That slowing down circulation and causes kidney hiperuricemia, what promotes the formation of crystals in the tubules and their obstruction. Pathogenesis of the glycerol ARF is complex and is not limited on violations of the crash syndrome. Important factors are renal ischemia to the hypovolemia, reduction in renal blood circulation, the decline in glomerular filtration, impaired ability to tubular reabsorption of sodium and water, development of hypernitrogenemia, significant renal protein loss, changes in antioxidant-proteolytic status. Massive rhabdomyolysis causes pronounced endotoxemia, indicating that poliorganic destruction.

In our experiments in rats glycerol ARF caused by single intramuscular injection of 50% aqueous solution of glycerol (10 ml/kg in muscle back legs once, dividing the dose equally between all the limbs)²⁶⁻²⁹.

To investigate the potential nephroprotective properties were elected sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid (PDT-Na) – drug with known anti-hypoxic action (corporation "Olifen", Moscow, Russia. The registry number RN№001939/02 07.10.2008, ATC code N07 XX).

The dose and method of administration – 90 mg/kg ones a day, during 14 days, inside stomach).

Hofitol as a reference drug (comparative), herbal agent with hypoazotemic and diuretic action (laboratory "Rosa-Phytopharma", Paris, France. ATC code A05A).

The dose and method of administration – 1.36 ml/kg ones a day, during 14 days, inside stomach).



According to the study design, the experimental animals were divided into groups (by 6 rats): 1st group - intact control; 2nd group - pathology (under the experimental conditions); 3rd group - pathology + PDT-Na (90 mg/kg); 4th group - pathology + Hofitol (1.36 ml/kg).

Doses determined according to the instructions for medical use of the considering a specific sensitivity factor according to Yu. R. Rybolovlev's method³⁰. All experimental rats had a standard diet regime, stable in renal disease development and for all time during the experiment^{1,2,8,9,23,24,26,27,29}.

To quantify the performance filtration and renal excretory function, characterizing the processes of glomerular filtration, tubular reabsorption and secretion in the nephron, conventional biochemical parameters studied. Urine was collected in special cages to separate the funnel-cell, investigated spontaneous daily diuresis. Blood was collected immediately after the decapitation of animals pursued under nembotal anesthesia (30 mg/kg intraperitoneally 0.1% solution).

In the collected urine and serum on the digital spectrophotometer PD-303 (Apel, Japan) according to the instructions used standard test set of reagents LLC "Spain-lab" (Kharkiv, Ukraine) determined the content of endogenous creatinine (kinetic method Jaffe), urea (kinetic method Urease-GLDH), total serum protein (biuret colorimetric method) and urine (photometric method for the reaction of sulfosalicylic acid).

On the data base, calculated parameters characterizing renal function in experiments, urine output per day and during minute, tubular reabsorption, glomerular filtration by clearance of endogenous creatinine, urea concentration ratio and clearance^{1,2,6-9,16,18,27,29,31}.

Research results were processed by variation biomedical statistics methods using t-Student criteria by computer program "Statistica 6.0".

RESULTS AND DISCUSSION

Under the experimental conditions in rats a single injection of 50% aqueous glycerol solution (10ml/kg intramuscularly) caused typical glycerol (mioglobinurical) ARF severe toxic kidney damage. Significant violations of filtration and renal excretory function is a direct consequence of the osmotic action of glycerol, which causes hypovolemia and decrease in renal blood circulation, and the direct impact of nephrotoxic massive intravascular hemolysis and rhabdomyolysis precipitation of myoglobin. Changes in tubules pressure in combination with the activation of vasoconstrictor factors in kidney provokes further falling of urine output and glomerular filtration.

In our experiments oliguric stage of glycerol ARF and fall in glomerular filtration process in nephrons during 14 days after administration of glycerol is characterized by

decreased urine output in 2.8 times and a significant decrease in glomerular filtration in 7 times.

Due to massive rhabdomyolysis of intracellular substances is release a broken nephrothelial structures and that changes function ability to tubular reabsorption. It shows a significant decrease of this index on 1.6% relative to intact control levels.

There is a severe disturbance of kidney nitrogen excretion function and development of uremia. Hypernitrogenemia characterized by the growth of creatinine and urea in serum in 1.5 times and in 7 times, respectively. Thus these indicators excretion in urine was significantly reduced by 38% and by 64% respectively compared to the intact group.

The severity of glycerol toxic kidney damage defined by a decrease in the concentration ratio of urea in 20 times, clearance and urea in 31 times relatively intact rats.

In experimental glycerol ARF critical violations filtration and renal excretory function and the development of proteinuria appears. In terms of spontaneous urination in our experiments found significant renal loss of total protein – content in the urine increases in 2 times, while reducing this index in serum in 1.3 times.

Table 1 showing on the revealed experimental data that the simultaneous introduction of PDT-Na (90 mg/kg one time a day, every day by 14 days) in rats with glycerol administration helps to restore impaired kidney function reliably normalizing all indicators studied.

Use of PDT-Na marked significant normalization of urine output, which exceeds in 2.9 times distinct to rate in rats with experimental disease, and even on 1.6% higher than the intact level, but on 14% lower than diuretic effect of referent drug Hofitol. Glomerular filtration under the influence of PDT-Na is also normalized (didn't reach the intact level by 18.6%), but in comparison with glycerol ARF increased almost in 6 times, and only on 2.7% less than similar effect of Hofitol.

The most significant diagnostic criterion of nephroprotective activity of experimental substances is considered to be reliable normalization effect on the stabilization of nitrogen metabolism. Effective prevention of myoglobin-induced uremia under PDT-Na use characterized in normalization of creatinine and urea excretion in the urine that appears to increase these parameters in 1.4 and in 2.2 times respectively, relatively to pathology.

The positive normalizing effect of PDT-Na also traced in dynamics of those products of nitrogen metabolism in blood serum. Content of creatinine decreased in 1.4 times, and the content of urea in 7 times compared with the level of pathology.



Table 1: Sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid influence on filtration-reabsorption and renal excretory function in rats in experimental glycerol acute renal insufficiency

Indexes	Experimental groups			
	Intact control	Pathology	Pathology + PDT-Na (90 mg/kg)	Pathology + Hofitol (1,36 ml/kg)
Urine Volume per day, ml	6,15±0,05	2,16±0,01*	6,25±0,03**/**	7,30±0,06*/**
Urine Volume, ml/min.	0,0043±0,00005	0,0015±0,00001*	0,0043±0,00002**/**	0,0051±0,00004*/**
Creatinine level in the blood, mcmol/l	69,57±4,56	103,25±6,32*	75,47±3,22**	83,58±4,53**
Creatinine level in the urine, mcmol/l	6903±394,0	4284±390,0*	6107±550,5**	5753±328,0**
Glomerular filtration, ml/min.	0,43±0,035	0,06±0,009*	0,35±0,023**	0,36±0,041**
Tubular reabsorption, %	98,98±0,085	97,44±0,335*	98,73±0,375**	98,51±0,157**
Urea in the blood, mmol/l	4,83±0,29	34,58±2,33*	5,01±0,34**/**	26,44±2,19*/**
Urea excretion mmol/l	24,76±1,82	9,03±1,24*	20,19±1,13**/**	11,34±0,9*
Urea concentration factor, units	5,32±0,64	0,27±0,02*	4,48±0,64**/**	0,45±0,07*/**
Urea clearance mmol/min.	0,345±0,0429	0,011±0,0019*	0,295±0,0423**/**	0,032±0,0047*/**
The total serum protein, g/l	67,20±0,49	52,00±2,47*	63,29±2,28**/**	53,27±1,64*
Total protein in the urine, g/l	0,16±0,03	0,34±0,06*	0,19±0,01**	0,26±0,06

Notes: * - difference relating to intact control ($p \leq 0.05$); ** - difference relating to pathology ($p \leq 0.05$); *** - difference relating to reference drug Hofitol ($p \leq 0.05$).

The concentration ratio and urea clearance on the background of PDT-Na were the most informative indicators of filtration and renal excretory function in experiments conducted, that is significantly normalized compared with pathology in 17 times and in 27 times respectively.

Table 1 showing that the hyponitrogenic effect of PDT-Na by all studied parameters show that nitrogen even surpasses well-known from the literature and observed in our experiments Hofitol effect: the content of creatinine in average by 6-10%, the dynamics of urea in 2-5 times and the concentration ratio and urea clearance in 9-10 times better than the comparator agent's level.

Research of nephroprotective action of PDT-Na confirmed by a reliable prevention of one of the fundamental signs of kidney failure - proteinuria, because the loss of protein in the urine is the most important diagnostic criterion of urine syndrome.

Proteinuria usually occurs due to damage of kidney filters and decreased ability of proximal tubular protein reabsorption in acute renal toxic lesions. In our experiments hypoproteinuric activity of PDT-Na characterized by decreased concentration of total proteins in the urine on 44% relatively with pathology and authentically equal to intact level, but on 27% better efficiency of Hofitol on this indicator.

Due to reliable normalization of tubular reabsorption, what on 1.3% higher than the level of disease and on 0.2% better results than that of reference drug Hofitol, total protein content in blood serum also normalized. This

parameter under use of PDT-Na on 22% higher than in glycerol ARF, and is better on 19% relatively to Hofitol.

Thus, in accordance with the goals and objectives, the results of the experimental study of PDT-Na impact on the performance of filtration-reabsorption and renal excretory function in rats in experimental glycerol ARF, proved what the two weeks use of this drug has nephroprotective properties much greater than those of reference drug Hofitol. That is supposed to be due to PDT-Na's nonspecific antihypoxic and antioxidant properties, what significantly normalizing all performance in this series of experiments.

CONCLUSION

Sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid (90 mg/kg by 14 days) use in experimental glycerol acute renal insufficiency in rats shows expressed nephroprotective properties.

Studied drug reliably prevents the development of severe toxic myoglobin-induced renal lesions represent with oliguria, hypernitrogenemia, proteinuria and uremia, diuresis and renal excretion of urea and creatinine normalized, glomerular filtration and tubular reabsorption recovers, protein loss eliminates.

Sodium poly-(2,5-dihydroxyphenilen)-4-thiosulfate acid is a promising object for the further experimental studying as an nonspecific nephroprotective agent.



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