Research Article

Pharmacological Study of New Urolitholytic Drug Flarosukcin in Experimental Immature Rats

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ABSTRACT

Flarosukcin influence on calcium and phosphorus homeostasis in experimental acute renal failure was studied in experimental Vistar immature rats weighing 40 - 50 g. Flarosukcin was administered in two doses: 2.0 ml / kg - effective dose determined in previous studies in cyclic rats, 3.2 ml / kg - dose considering a dose factor corresponding to the age. The drug is proved to be focused upon the recovery of calcium and phosphorus excretion with urine. Farosukcin contributed to the physiological growth of animal body weight and preserved kidney mass index. Thus, Flarosukcin demonstrated more effective pharmacological properties in a dose of 2.0 ml / kg and had considerable advantages over comparator Canefron N.

Keywords: Flarosukcin, calcium and phosphorus homeostasis, urolithiasis, experimental renal failure, comparator Canefron N.

INTRODUCTION

Urolithiasis tends to be increased in many countries, including Ukraine, predicted to the further growth due to recently changed diet nature and quality (consumption of stabilizers, preservatives, coloring agents, protein food, salt, low daily water consumption, etc.), increased quantity of ecology and social factors affecting directly and indirectly human health.

Urolithiasis relevance is caused by the fact that on the average - 60% diagnosed in able-bodied people of 20-55 age. It should be noted that recently urolithiasis is increasingly observed in children. The conducted studies (E. V. Cherepanova 2008) allowed to allocateurolithiasis risk factors in a pediatric group:

- A family history of risk factors of urolithiasis, urological diseases, especially accompanied by metabolic disorders in stone-forming substances - 68.5% of cases;
- Anomaly in the urinary system - 21.2% of cases;
- living in ecologically unfavorable conditions - 88.5% of cases;
- Parents' bad habits (smoking, alcohol abuse) - 16.6% of cases;
- mother's burdened pregnancy - toxicity (82.7% of cases), intake of antiviral and antibacterial drugs (16.6% of cases).

Kidney disorder due to severe metabolic disorders: hyperoxaluria (8,1-32%) and hyperuricuria (23-35.8%), hypocitraturia (28-44.3%), hypomagnesaemia (6.8-19%) hypercalcemia (36.7-60.9%) of various genesis are combined into a dismetabolic nephropathy group.[1-3]

Dismetabolic nephropathy common feature is believed to be predominant disorder of the renal interstitium with deposited salts and the further development of fibrosis, disordered trophism of renal tubules and etc. Increased excretion by the kidneys of disordered metabolic products as crystals or conglomerates damages intracellular structures of the epithelial cells of the renal tubules, worsening reabsorption and secretion. Severe crystalluria often leads to concrement formation.

However, in some cases, in patients with urolithiasis no metabolic disorders of lithogenic character are revealed. According to authors, the share of such patients is quite significant from 11 to 36%.[4-10]

In addition, hypercalciuria is not revealed in 50% as one of important lithogenesis risk factor.[1, 11, 12]

These observations can be associated with urine glycoproteids able to modulate the calculus formation. These substances are excreted in the urine by tubular epithelial cells and able to accelerate or inhibit the crystal nucleation, growth and aggregation.[13, 14]

They include the Tamm-Horsfall glycoprotein, glycosaminoglycans, uropontine, nephrocalcine, bukinin. All these compounds produced by the renal urothelium are excreted into tubule lumen and in a varying degree included in the crystal matrix.[15-17]

Macromolecule effect on lithogenesis is quite complex and is actively investigated.

Crystalline components of urinary stones are formed from uric acid, oxalic acid, and phosphoric acid in the urine in certain hydrogen ion concentration for each stone, with the appropriate urine pH, this index is a leading risk factor for kidney stones.

Conservative treatment of urolithiasis and dismetabolic nephropathy should be focused to eliminate etiological factors and obstruction of urine flow.

The combined treatment includes drugs of different pharmacotherapeutic groups. The conservative urolithiasis therapy includes drugs normalizing urinary pH, dissolving stones, anti-inflammatory, antibacterial and analgesic drugs. To success this therapy should be systematical and long. It uses herbal drugs.[18-20]

Drug plants differ by chemical compound and contain a large number of biologically (pharmacologically) active substances. The spectrum of drug plant biological activity is determined by sufficient substances of different chemical classes and groups available in almost every drug plants (essential oils, flavonoids, polyphenols, polysaccharides, etc).

Biologically active substances of drug plants have a diuretic, antispasmodic, antimicrobial, membrane-stabilizing, nephroprotective and other actions. For example, Canephron N, Chophytol, Lespenephril and etc are widely used in the kidney and urinary tract treatment in children.

In addition, biologically active compounds of drug plants reduce proteinuria, normalize nitrogen metabolism. Thus, frequently, in spite of the diuretic effect of herbs, they are able to restore the ionic balance.[21-25]

Thus, drugs to treat kidneys and urinary tracts in children should have the following types of actions: to normalize protein, nitrogen and electrolyte metabolism, to maintain urine pH within a physiological range of values preventing lithogenesis, to restore glomerular filtration and tubular reabsorption, and to show antispasmodic and diuretic activity, reducing the urinary tract obstruction and increasing decay product excretion.

The aim of our research was to investigate the effect of new original drug Flarosukcin ("JSC SPC "Borschagivsky Pharmaceutical Plant", Kiev, 120207 Series) on electrolyte metabolism in immature animals with experimental renal failure.

MATERIALS AND METHODS

Flarosukcin was previously studied and found to show a significant effect, to normalize renal function in experimental cyclic rats.[26,27]

Its influence on the experimental renal failure in immature animals was of interest to investigate.

CanephronN solution (Bionorica, Germany, 0705001738 series) common for pediatric nephrology was used as a comparison drug.

The animal use for scientific and research purposes provides humane care of animals (Law of Ukraine "On the Prevention of Cruelty to Animals," 2006, European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes, Strasbourg, 1986).

All studies were conducted in accordance with modern scientific standards, provided all ethical aspects of the humane care of animals in researches (report of the National Pharmacy University Bioethics Committee No.11 of 16.11.2011).

Experimental studies were conducted in Vistar immature albino rats of both genders, aged from 0.5 to 1 month, which corresponds to 2-7 year old children, weighing 40-50 g. The animals had access to water and were kept on a standard diet.

The experimental renal failure was modeled by a single intramuscular injection of 50% glycerol solution at 10 ml / kg ("glycerol" model of acute renal failure). The literature reports that a single intramuscular injection of the said glycerol dose in animals causes oliguria, decreases glomerular filtration and tubular reabsorption, develops azotemia. These disorders are associated with development of renal cortical ischemia, swelling of the convoluted tubule epithelium, function disorder.[28, 29, 30]

Investigated Flarosukcin was administered to the animals intraperitoneally daily by gavage for 14 days. This period of time was selected basing on the literature, testifying that the maximum changes in renal function in experimental renal disease were developed to the 14-16th day, then by the 21-24th day changes were decreased, but by the 28-30th day - renal function was almost completely normalized.[31, 32]

Flarosukcin was administered in immature animals in two doses: 2.0 ml / kg - effective dose determined in previous studies in cyclic rats, 3.2 ml / kg - dose considering a dose factor corresponding to the age.[33]

Comparator Canephron N was administered to the animals in a dose of 1.0 ml/kg. A comparison drug dose is defined according to the leaflet taking into account the species sensitivity index by R. Rybolovlev.[34]

The animals were divided into 5 groups (n = 8): 1 - intact animals; 2 - control pathology (intramuscular injection of 50% glycerol solution), 3 - pathology + Flarosukcin in a dose of 2,0 ml / kg; 4 - pathology + Flarosukcin of 3.2 ml / kg; 5 - pathology + Canephron N in a dose of 1.0 ml / kg.

Influence of the investigated drug on calcium and phosphorus homeostasis was studied by values of the calcium and phosphorus content in blood and daily urine using the standard set "Felicit-Diagnosis" (Ukraine).

Once the experiment was completed, the animals were humanely euthanatized with thiopental anesthesia. The kidney was weighed followed by the mass index calculating.

Statistical analysis was made using the statistical software «Primer Biostatistics», «Sigmastat» (USA, 1994). Statistical significance of intergroup differences was evaluated using Student's t-distribution. Changes were considered as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Flarosukcin influence on calcium homeostasis

The kidneys are known to play a key role in the calcium metabolism. Renal failure is characterized by a decrease in glomerular filtration rate and volume of filtered liquid, in this connection calcium excretion by the kidneys is disordered.

The study revealed (Table 1) that a single intramuscular injection of 50% glycerol solution resulted in a slight decrease in blood calcium by 14.5% in the pathology control group comparing to the intact animals, however, were within the physiological range. At the same time, daily calcium excretion was decreased. Calcium in immature rats' urine in this group was reduced comparing to the intact control by 1.8 times.

Flarosukcin administration in a dose of 2.0 ml / kg in pathology development maintained the calcium concentration in the blood at the intact group level and at the same time increased the calcium excretion through the kidneys at the intact group level.

Flarosukcin administration in a dose of 3.2 mg / kg also maintained the calcium concentration in the blood at the intact group level, but calcium was more intensively excreted through the kidneys - by 22% higher than at the intact control group.

Comparator Canephron N in contrast to Flarosukcin does not increase urinary calcium excretion through the kidneys in experimental renal failure. The calcium level in the urine at this group was by 1.8 times lower than at the intact control by the 14th day of the experiment.

Thus, the experimental renal failure development caused hypercalciuria. Investigated Flarosukcin in both doses contributed to increased calcium excretion. Thus, Flarosukcin dose of 3.2 ml / kg more intensively excretes calcium comparing to a dose of 2.0 ml /kg, which may lead to the hypercalciuria development.

Comparator drug did not restore the daily calcium excretion.

Flarosukcin influence on phosphoric homeostasis

Calcium and phosphorus exchange is interconnected. Increased calcium intake with food is accompanied by decreased phosphorus excretion in the urine. Increasing the blood phosphorus concentration is apparently caused by the hypercalcemia effect releasing intracellular phosphorus into the bloodstream. At the same time, hypercalcemia can directly affect the kidneys, increasing the tubular phosphorus reabsorption regardless of parathyroid hormone. Restoration of the physiological blood calcium level in the patients with hypoparathyroidism leads to increased phosphorus excretion in the urine. A significant phosphorus inflow in the experimental animals after parathyroidectomy induces a decrease in tubular phosphorus reabsorption, associated with concomitant decreased calcium concentration in the blood. Therefore, maintaining a constant calcium level can compensate disordered phosphorus reabsorption. The high catabolism with increased tissue destruction and the metabolic acidosis are associated with hypophosphaturiatypical for renal failure. [35]

Thus, the renal insufficiency development is characterized by changes in blood or urine, both of calcium and phosphorous. Therefore to study Flarosukcin influence on the phosphorus balance in immature animals with experimental renal failure was of interest.

According to Table 2 data the phosphorus content in the pathology control animals' blood was not increased and was within physiological range. A daily urinary excretion was reduced by 2.2 times comparing to the intact control, i.e. the experimental renal insufficiency development was accompanied by hypo phosphaturia.

Flarosukcin in a dose of 2.0 ml /kg in the pathology reduced blood phosphorus level to the intact values and increased the daily excretion comparing to the pathology control by 2.3 times that corresponded to the intact values.

Flarosukcin in a dose of 3.2 ml /kg increased the phosphorus excretion through the kidneys by 2.7 times comparing to the pathology group and by 24% more intensively comparing to the intact control.

Comparator Canephron N administered in renal failure showed a similar effect, maintained the blood phosphorus at the intact control level. However, phosphorus excretion in urine was not restored, which correlated with the data obtained at the study of the drug influence on the urinary calcium excretion.

Table 1: Flarosukcin influence on calcium level in experimental immature rats with experimental acute renal failure

Groups of animals	Dose, ml/kg	Calcium in the blood, mmol/l	Calcium excretion mmol /day
Intact control (n=8)	–	2.70 ± 0.24	77.44 ± 9.55
Pathology control (n=8)	–	2.31 ± 0.14	42.96 ± 10.80*
Flarosukcin (n=8)	2.0	2.56 ± 0.15	76.28 ± 9.39**/***
Flarosukcin (n=8)	3.2	2.50±0.07	94.23 ± 14.02**/***
Canephron N (n=8)	1.0	2.50 ± 0.15	43.92±8.10*

* - difference reliability relating to intact control (p≤0.05); ** - difference reliability relating to pathology control (p≤0.05); *** - difference reliability relating to comparative drug (p≤0.05).

Table 2: Flarosukcin influence on phosphorus level in experimental acute renal failure in experimental immature rats

Groups of animals	Dose, ml/kg	Phosphorus in the blood, mmol/l	Phosphorus excretion mmol /day
Intact control (n=8)	–	2.48 ± 0.103	142.10 ± 19.20
Pathology control (n=8)	–	2.60 ± 0.058	64.45 ± 20.30*
Flarosukcin (n=8)	2.0	2.43 ± 0.081	147.24 ± 11.10**/***
Flarosukcin (n=8)	3.2	2.40±0.072	176.36 ± 18.67**/***
Canephron N (n=8)	1.0	2.47 ± 0.072	59.27±12.10 *

* - difference reliability relating to intact control (p≤0.05); ** - difference reliability relating to pathology control (p≤0.05); *** - difference reliability relating to comparative drug (p≤0.05).

Table 3: Changes in body weight and kidney weight ratio when using Flarosukcin

Groups of animals	Dose, ml/kg	Animal body weight, g		Kidney weight ratio, %
		initial	on the 14th day	
Intact control (n=8)	–	47.14±1.84	80.70±2.02*	0.246±0.009
Pathology control (n=8)	–	46.25±1.57	55.63±2.58*	0.600 ± 0.056
Flarosukcin (n=8)	2,0	46.88±0.92	75.00±1.63**/***	0.243±0.01* */* * * *
Flarosukcin (n=8)	3,2	44.38±4.93	66.88 ± 1.87*/**	0.318 ± 0.013*/* *
Canephron N (n=8)	1,0	45.00±1.60	65.50 ± 2.32*/**	0.326 ± 0.02*/* *

* - difference reliability relating to intact control (p≤0.05); ** - difference reliability relating to pathology control (p≤0.05); *** - difference reliability relating to comparative drug (p≤0.05).

Flarosukcin influence on body weight gain in immature rats and Kidney weight index in experimental renal failure

Based on the study results presented in Table 3, the pathology control animals' body weight was decreased on the 14th day of the experiment by 31% comparing to the intact animals. Kidney weight ratio was increased by 2.4 times, indicating the renal disease development.

Flarosukcin administration in a dose of 2.0 m /kg in pathology increased the animals' body weight by 60% and almost reached the intact control values. Kidney weight ratio was not proved to differ from the intact control.

Flarosukcin administration in a dose of 3.2 ml /kg increased animals' body weight by 51%. However, did not reach the intact animals' values - by 17% less. Kidney weight ratio was decreased comparing to the pathology control by 1.9 times, but did not reach intact animals' values, exceeding by 29%.

Comparator Canephron N also increased the animals' body weight by 46%, relatively to baseline, but did not reach the intact group values. Kidney weight ratio when using the comparator drug was decreased comparing to the pathology group by 1.8 times, but did not reach the intact group values, exceeding by 33%.

CONCLUSION

1. Hypercalciuria and hypo phosphaturia are observed in experimental "glycerol" acute renal failure.
2. Flarosukcin provides a positive effect on calcium and phosphorus homeostasis in experimental renal failure, shows a pronounced protective action, provides the physiological body weight gain in animal and preserves the kidney weight ratio.
3. Flarosukcin in a dose of 2.0 ml /kg has more pronounced effect reliably exceeding the comparative drug action.

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