THE INFLUENCE OF INDOLINOREN ON KIDNEY FUNCTION IN CONDITIONS OF WATER AND SALT LOAD

© A. Markina, O. Mishchenko

1. Introduction

The close relationship between water-salt metabolism and homeostasis is determined by the evolution of living organisms [1]. With the development of civilization, a large number of people got free access to fine salt. It is this that brought the human body to new, unusual conditions of sodium overload. The high proportion of canned products in the structure of modern nutrition, stable behavioural reactions and socio-cultural traditions, the complexity of quantitative control of sodium in the daily diet, complicate its limitations in physiologically acceptable consumption standards [2]. Today, there is no doubt that increased consumption of cooked salt is one of the causes of the spread of arterial hypertension and disruption of the normal functioning of the kidneys. The value of excess sodium increases with a simultaneous decrease in potassium levels [3]. In turn, the low-sodium diet can negatively affect mechanisms of regulation of water-salt homeostasis, activating the system of delayed sodium, especially the renin-angiotensin-aldosterone system [3].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Violation of water-electrolyte balance is the leading link in the pathogenesis of many diseases (kidney disease, disruption of the cardiovascular system, etc.) [4, 5]. Kidneys as one of the components of the functional system of water-salt metabolism provide maintenance of the main constants of the internal environment of the organism of animals and humans, modulating the state of its functional reserves and mechanisms. The tension of the systems of regulation of water-salt balance creates preconditions for the development of renal failure [4]. Warning and correction of water-electrolyte and metabolic disorders are among the tasks of medical therapy, along with the restoration of intravascular volume [6]. Diuretics are one of the groups of drugs used to correct kidney disorders and to establish their functional balance [6]. In turn, the ionic composition of foods (to a greater extent, sodium ions), which directly affects the excretory function of the kidneys, may act as a modulator of diuretic activity [3].

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

Today, the data concerning the modulation of the diuretic effect of the diuretic salt regimen are quite controversial. In particular, R. Babini et al. [7] found that in rabbits at a decrease in salt content in the diet, the diuretic effect of furosemide increased. According to another data [3], with prolonged (1 month) consumption of sodium chloride in rats, the saluretic efficacy of furosemide and dihydrochlorothiazide in a single dose is somewhat reduced.
4. Formulation of goals (tasks) of article

The purpose of this work was to determine the peculiarities of the effect of a new acylated derivative of propyl ester N - [(2-oxoindolinylidene-3) -2-oxaacetyl] valine – leader on diuretic activity among esters derivatives of N - [(2-oxoindolinylidene-3) 2-oxaacetyl] amino acids, to the condition of excretory renal function under the conditions of water and salt loading. The new compound already has diuretic activity [8] and the ability to improve excretory renal function in acute glyceryl renal insufficiency [9].

5. Presentation of the main research material (methods and objects) with the justification of the results

Materials and methods of research. The object of the pharmacological study was propyl ester N - [(2-oxoindolinylidene-3) -2-oxaacetyl] valine (conventional name "Indolinoren"), synthesized at the Department of Analytical Chemistry of NUPH under the direction of prof. S. V. Kolesnik.

Experiments were conducted on 60 non-linear rats weighing 200 ± 20 g. Animal care was carried out in accordance with the Law of Ukraine No3447-IV "On the protection of animals from cruel treatment" [10] and in accordance with the EU Directive of 10/10/2010 on the protection of vertebrates animals used for experimental and other scientific purposes [11].

Water loading (WL) was modelled by the introduction of distilled water at a rate of 5 ml per 100 g body weight of animals [12]. Salt loading (SL) was reproduced by intragastric administration to animals 0.45 % solution of sodium chloride in the amount of 3 % of body weight [13].

Experimental animals were divided into 6 groups (10 animals per group): group I – intact control for WL groups; group II – rats with WL treated with indolinoren; III – rats with WL treated with the comparator preparation – furosemide (produced by the ZAT NVC "Borschagovsky KhFZ", Ukraine); IV – intact control for groups SL; V – rats with SL, which were given indolinoren; VI – rats with SL, which were administered a comparison drug furosemide.

Indolinoren was administered once intravenously in a conditionally effective dose of 29.5 mg / kg, which was determined during previous studies with diuretic activity, in the form of a fine aqueous suspension stabilized by a tween-80, half an hour before loading. Furosemide was administered in the same mode of intragastric administration at a dose of 5 mg / kg as an aqueous solution [3]. Within 2 hours after the introduction, the animals were in the individual exchange cages for collecting urine. The volume of urine was measured. The excretion rates of creatinine, sodium, and potassium were also determined. Determination of the concentration of creatinine in blood plasma and urine was carried out with the help of laboratory kits "Filisit-diagnostics" [14]. The concentration of sodium and potassium in urine and blood plasma was measured by the method of flame photometry [15]. Calculations of excretion of electrolytes were carried out according to generally accepted formulas [12]. All received material was statistically processed using parametric statistical methods [16].

6. Results and discussion

Analysis of the data showed that in the conditions of WL, indolinoren increases urinary excretion by 167 % relative to intact control (p <0.05) (Tab. 1). Under conditions of WL, indolinoren exhibits a saluretic effect, which was accompanied by an increase in excretion of sodium by 132 % and potassium by 2.4 %, which may indicate a decrease in their reabsorption in the tubules of the nephrons. There was no significant difference in creatinine excretion rates, which may indicate that indolinoren has no effect on the velocity of glomerular filtration. A significant increase in the sodium-potassium urine ratio was established against the background of the introduction of indolinoren and the furosemide comparison drug in 2.3 and 2.1 times, respectively, with regard to intact control (p <0.05), which indicates a more distinct natriuresis than kaliuresis is in the background of their action [17]. There was no significant difference in the diuresis value in rats given the indolinoren and the furosemide comparison preparation (Table 1).

Influence of indolinoren on diuresis and electrolyte excretion under the conditions of water and salt load

Table 1

| Conditions of experiment | Diuresis for 2 hours, ml / 100 g | Excretion, μmol /100 for 2 hours | (X ± Sx, n=10) |
|--------------------------|-------------------------------|----------------------------------|----------------|
|                          |                               | creatinine                      | sodium         | potassium     | Na /K coefficient |
| Water loading (WL)       |                               | 3.65±0.19                       | 53.95±2.10     | 80.56±3.15   | 0.67±0.06       |
| Intact control           | 1.95±0.56                     | 3.65±0.19                       | 53.95±2.10     | 80.56±3.15   | 0.67±0.06       |
| Indolinoren, 29.5 mg/kg  | 5.20±0.50*                    | 3.70±1.10                       | 125.10±6.20*   | 82.50±2.40** | 1.51±0.04*      |
| Furosemide, 5 mg/kg      | 5.80±0.30*                    | 4.10±1.40                       | 127.00±6.40*   | 89.40±2.10*  | 1.42±0.05*      |
| Salt loading (SL)        |                               | 3.61±0.14                       | 61.95±1.25     | 80.45±3.10   | 0.77±0.05       |
| Intact control           | 1.85±0.32                     | 3.61±0.14                       | 61.95±1.25     | 80.45±3.10   | 0.77±0.05       |
| Indolinoren, 29.5 mg/kg  | 8.90±0.8*/<>/                  | 3.60±0.50                       | 140.65±5.90*   | 86.05±2.94   | 1.63±0.04*      |
| Furosemide, 5 mg/kg      | 6.10±0.41*                    | 4.30±0.63                       | 155.80±4.20*   | 89.80±3.10   | 1.73±0.05*      |

Notes: * – deviation is significant relative to intact control, p <0.05; ** – deviation is significant relative to furosemide, p<0.05; @ – deviations are reliable relative to the corresponding indicator in the group WL, p <0.05; n – the number of animals in the group

Under conditions of SL in the intact control group, increased sodium excretion was noted and no significant changes in the creatinine excretion index were noted. The removal of potassium and water was almost
the same as with WL. The obtained data coincide with the data of the literature, which indicate a delay in diuresis in dogs for the second hour after SL with 0.4 % solution of sodium chloride [12]. It is known that in the study of excretory kidney function by the method of daily urine collection, in most animals receiving SL, the excretion of water and osmotically active substances increases in parallel [3]. Therefore, the lack of expressive diuresis in intact rats after SL in our experiment may be due to a relatively short time interval for urine collection (2 hours).

Under conditions of SL, indolinoren contributed to a significant increase in urine output by 381 % (p < 0.05), increased sodium excretion by 127 % (p <0.05), and potassium by 7 %. The drug comparison of furosemide in high-sodium diet resulted in an increase potassium and sodium by 11 % and 151 %, respectively, and contributed to a 300 % increase in diuresis (p <0.05) relative to intact control, that is, inferior to indolinoren in diuretic activity (p <0.05). In contrast to the data obtained in our experiment, in the work of H. Herken (1961, cit. by E. B. Berhine, 1967), an increase in the effectiveness of diuretic action of furosemide in rats under conditions of SL was established. The difference in the results may be related to the conditions of the experiment. In experiments, H. Herken administered intraperitoneally 0.9 % sodium chloride solution for 24 hours to rats. The rats in our experiment were administered a hypotonic solution of sodium chloride once [7]. On the background of both agents, no reliable changes in the creatinine excretion index were established (Tabl. 1). There was a significant increase versus the group of intact control of sodium-potassium ratio in 2.1 times under the influence of indolinoren and 2.3 times in the background of furosemide action, which indicates a more pronounced growth of natriuresis than kaliuresis, and reflects a decrease in mineralocorticoid control of excretory renal function, that is anti-aldosterone effect [3].

Consequently, under conditions of SL, the diuretic and saluretic activity of indolinoren at a dose of 29.5 mg/kg increases and prevails the relevant parameters in conditions of WL. An increase in natriuresis and, to a lesser extent, kaliuresis in the background of the introduction of indolinoren in the conditions of WL and SL suggests that the diuretic effect is realized through the inhibition of the tubular transport and practically does not affect glomerular filtration, since the values of excretion of creatinine acting as a marker of glomerular filtration remained at the level of output indicators.

The obtained data concerning the enhancement of indolinoren effects under the conditions of SL is an important feature in terms of the prospects for the use of propyl ester of N(2-oxoindolinylidene-3)-2-oxacyethyl-valin as a diuretic in patients both in normal diet and in excess salt.

7. Conclusions from the conducted research and prospects for further development of this field

1. According to the data of the study of the effects of propyl ester N(2-oxoindolinylidene-3)-2-oxacyethyl-valin under the conditional name "Indolinoren" on the functional state of the kidneys under the conditions of water and salt loading, a clear diuretic and saluretic activity was established.

2. The new study compound indolinoren (29.5 mg/kg intragastric) with diuretic activity under salt loading dominates in comparison with furosemide (5 mg / kg intragastric) and does not have a significant difference in the investigated parameters under water loading conditions.

3. Increase in natriuresis and to a lesser degree of kaliuresis and absence of probable changes in excretion of creatinine - a marker of glomerular filtration - against the background of the introduction of indolinoren under the conditions of water and salt loading gives reason to suppose that its diuretic effect is realized through the inhibition of tubular reabsorption, and an increase in urine sodium-potassium coefficient indicates a decrease in mineralocorticoid control of excretory renal function. The obtained data are the basis for further in-depth study of the compound "Indolinoren" as a perspective diuretic agent.

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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF MELDONIUM DIHYDRATE IN DOSAGE FORMS

© A. Donchenko, N. Nahorna, S. Vasyuk

Meta. Спектрофотометрія є одним із найбільш широко застосовуваних методів у фармацевтичному аналізі. Висока чутливість, економічність та доступність для більшості лабораторій контролю якості лікарських засобів є безперечно перевагами цього абсорбційного методу. Однак існує необхідність пошук у нових аналітичних реагентів для розробки методу кількісного визначення. Таким чином робота стало дослідження взаємодії мельдонію дигідрату з п-хлоранілом та розробка на основі отриманих даних спектрофотометричної методики аналізу препарату в лікарських формах.

Методи. В дослідженні використовували робочий стандартний зразок мельдонію дигідрату, п-хлораніл, ДМФА, зразки готових лікарських форм. Для визначення оптичної густини продукту реакції застосували спектрофотометр Specord 200.

Результати. В ході розробки методики були підібрани оптимальні умови проведення спектрофотометричного аналізу. Досліджено вплив на перебіг реакції таких чинників як природа розчинника, концентрація реагенту, температура та час нагрівання. Експериментально встановлено, що мельдоній взаємодіє з п-хлоранілом у середовищі ДМФА з утворенням забарвленого сполку з максимумом абсорбції при 556 нм. Проведено валидацию розробленої методики. Визначено основні валидационні характеристики, а саме, лінійність, прецизійність, правильність, робочість та діапазон застосування. Підпорядкування закону Бера спостерігається в межах концентрацій 8.00-20.00 мг/100 мл, коефіцієнт кореляції становить 0.9995. Параметри лінійної залежності розраховано за допомогою регресійного аналізу методом найменших квадратів. Запропонована методика відповідає вимогам ДФУ, які висувають до методик кількісного аналізу лікарських речовин.

Висновки. Розроблено та валидовано спектрофотометричну методику кількісного визначення мельдонію дигідрату, яка успішно застосована для аналізу лікарських форм. Результати дослідження свідчать, що методика є точкою, простою у виконанні та пригодною для використання в лабораторіях контролю якості лікарських речовин.

Ключові слова: спектрофотометрія, похідний хіноку, п-хлораніл, мельдонію дигідрат, аналіз, кількісне визначення, валидация

1. Introduction

Today, metabolic therapy is an important component of the treatment of virtually any disease of the internal organs. Drugs affecting the metabolic processes in the heart, brain, liver, muscles, are widely prescribed by general practitioners and narrow specialists. A special place among them take cardioprotectors – a group of drugs that improves metabolic processes in ischemic myocardium, increase resistance to hypoxia, eliminate cellular metabolism disorders. To the well-known and recognized by clinicians cardioprotectors also belongs meldonium [1]. The drug was created at the Latvian Institute of Organic Synthesis in the mid-1970s and was used as a veterinary product. In clinical practice meldon-