THE IMPACT OF INDOLINOREN ON HISTROSTRUCTURE OF KIDNEY ON THE GLIXERROID ACUTE RENAL FAILURE MODEL

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1. Introduction

According to statistics, the frequency of acute renal failure syndrome (ARF) is 30–50 cases per 1 million population per year, and in one of them it is necessary to use hemodialysis for treatment [1]. In the structure of nephrological diseases, ARF takes 10% of cases. The ARF may be accompanied by various complications, sometimes causing death, but it is one of the few syndromes in which complete recovery of the organ function can be achieved [2, 3]. Mortality of patients with ARF ranges from 0.2 to 2% [4, 5].

2. The urgency of the topic and its relevance to important practical issues

The urgency of the problem of ARF caused, firstly, by the widespread causes of its occurrence and severe complications, and secondly, the lack of noticeable progress in the treatment and prevention of this syndrome in recent years. In developed countries, the number of cases of ARF associated with polypharmacy and sensitization to drugs has increased by 6–8 times over the past 10 years. The frequency of post operative acute renal and multiple organ failure, which is associated with an increase in the complexity of surgical interventions, has also grown [3]. An increase in the number of cases of transient neuralgia in the terminal stage of chronic renal failure has been noticed too.

3. An analysis of recent researches and publications on the solution of this problem

Diuretics are widely used in the treatment of ARF, in particular to exclude the oliguria, but may provide a number of side effects [4]. Therefore, the search for new safe medicines that can positively affect on the course of ARF is one of the important tasks of experimental pharmacology.

The analysis of literary sources has shown that derivatives of 2-oxindoline show diverse pharmacological activity, in particular diuretic, and therefore they are perspective for the search and creation on their basis highly effective and low-toxic preparations with diuretic action [6].

4. Formulating of the task

The purpose of this work was to study the effects of the new derivative 2-oxindoline with the conditional name “Indolinoren”, for which diuretic activity was established [6] and the ability to improve the renal excreto-
ry function under the conditions of the ARF [7] on histologic structure of kidney of rats under the conditions of this pathology.

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

Materials and methods of research. The subject of the pharmacological study is the compound (the conditional name “Indolinoren”), which is the propyl ester N-(2-oxindolinylidene-3)-2-oxacetyl valine (Fig. 1) synthesized at the department of analytical chemistry of NPhU under the guidance of prof. Kolisnyk S.V.

![Fig. 1. Chemical structure of propyl ester N-(2-oxindolinylidene-3)-2-oxacetyl valine – compound “Indolinoren”](image)

For the study, was used the compound in the conditionally therapeutic dose of 29.5 mg/kg for diuretic activity, which was established during the previous studies.

In the experiment was used the glycerol model of ARF, which is reproduced by intramuscular administration to the test animals of 50% solution of glycerol in a dose of 10 ml/kg once [8]. Experiments were performed on 32 white non-linear rats weighing 180–220 g. The performed researches do not contradict generally accepted bioethical norms, carried out in compliance with the relevant international regulations on conducting experimental research [9]. Experimental animals were divided into 4 groups (8 animals per group): group I – intact control; group II – rats with ARF without treatment (control group); III – rats with ARF, which received “Indolinoren” in the conditional dose of 29.5 mg/kg, in the form of a fine-dispersed aqueous suspension stabilized by a tween-80; IV – rats with ARF, which was administered with the comparator – furosemide (produced by the PJSC SIC “Borschchivskiy CPP”, Ukraine) at a dose of 5 mg/kg as an aqueous solution, which is included in the treatment protocols of the ARF in the clinics [10].

The test compound and furosemide were injected intragastrically in the therapeutic prophylactic mode for 3 days before the ARF simulation and within 2 days in the background of ARF. Sacrifice of animals of all groups was carried out 72 hours after the injection of glycerol. Samples of the kidneys were fixed in 10% formalin solution, dehydrated in spirits of increasing concentration, and embedded in paraffin. Sections of 4–5 μm were cut and stained with hematoxylin and eosin [11]. Sacrifice was performed by overdose of ethereal anesthesia. On the sections, was performed a semi-quantitative assessment (in points) of the state of the tubular apparatus of the nephrons (the most vulnerable in this pathology) on the following morphological parameters: the severity of necrosis, vacuolar dystrophy of the nephrocytes, the presence of cylinders in the lumen of the tubules [11]. The evaluation results were presented in points: 0 points – no changes; 1 point – weak changes (affecting 10–15% of tubules); 2 points – moderate changes (affect up to 25% of tubules); 3 points – expressive changes (affect up to 50% of tubules); 4 points – very distinct changes (affect up to 90% of tubules).

All received digital material was statistically processed using a nonparametric analogue of one factor analysis – the Kruskal-Wallis criterion, and then the Man-Whitney’s criterion with Bonferoni’s correction (the standard statistical software package “Statistika” 5.0, 6.0) [12] was applied. Microscopic study of micropreparations was performed with a microscope Micros 400. Images were taken with a NiconColPix 4500 digital camera. Pictures were processed on a Pentium 2.4 GHz computer using NiconView 5.

Results and discussion

At the time of sacrificing the animals, in all experimental groups that were observed survival rate was at 100%.

During the histological study of kidney structure in all the rats in the control group was observed necrosis of the distal and proximal parts of the tubules of the nephrons with complete violation of the tissue pattern. The basement membrane of most necrotic tubules was destroyed. The accumulation of eosinophilic amorphous masses and cylinders can be seen in the lumen of the tubules of the cortico-medulla zone, the collecting ducts and the straight tubules of the renal medulla, as well as in the few preserved convoluted tubules.

![Fig. 2. Rat kidney with a model of glycerol acute renal failure for 3 days after the administration of glycerol: a – necrosis of the distal and proximal parts of the tubules of the nephrons, vacuolar dystrophy of the nephrocytes of isolated preserved tubules; b – destruction of basement membranes (arrows) of necrotic tubules. Hematoxylin-eosin. × 200, × 400](image)
On the background of administration of the compound "Indolinoren", was noted improvement of the tubal system of rat’s nephrons. The necrotic changes were minimized in 4 out of 8 rats, there was no disruption and distortion of the nephrocytes of the distal and proximal parts of the tubules of the nephrons, visually reduced the number of tubules in the cortico-medullary zone and the cerebellum layer, the lumen of which was blocked by cylinders (Fig. 3).

Fig. 3. Rat kidney with a model of glycerol acute renal failure with the introduction of "Indolinoren" on the 3rd day: a – single-substituted convoluted tubules (arrows) on the background of intact; b – reduction of cylinders in the channels of the cortico-medulla zone. 
Hematoxylin-eosin. ×200

The condition of the vast majority of renal glomeruli of all rats in this group practically did not change, except for individual glomeruli (Fig. 4).

After the administration of the drug comparison furosemide only one of the 8 rats had normal condition of the tubular renal system. The rest of the animals that was observed had dystrophic-necrotic changes of the distal and proximal parts of the tubules of nephrons, ranging from weak (0.38 points) to expressive (2.38 points) (Fig. 5).

Fig. 4. Rat kidney with a model of glycerol acute renal failure on the background of the administration of "Indolinoren" on the 3rd day: a – kidney glomerulus corresponds to the norm; b – densely shaped kidney glomerulus, the pattern of capillary loops is not visualized. Hematoxylin-eosin. ×400

The data of the semi-quantitative assessment of the condition of the tubular apparatus of the nephrons also indicate that on the model of glycerol ARF, the compound “Indolinoren” had a strong nephroprotective effect, significantly reducing the pathological changes in the system of tubular nephron compared to the control pathology (Table 1), reducing the severity of necrosis, vacuolic dystrophy and obstruction the lumen of the tubules by cylinders in 1.7 times. There is no reliable difference between the indicators for animals with ARF under the influence of the compound “Indolinoren” and furosemide comparison drug. Consequently, the new investigational compound “Indolinoren” on the severity of the normalizing effect on the histostucture of kidneys of rats with ARF is not inferior in compare to the loop diuretic furosemide.

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Fig. 5 Rat kidney with a model of glycerol acute renal failure on the background of the administration of furosemide on the 3rd day. Fluctuations in the severity of cortical tubular disorders: a – weakly expressed (0.38 points); b – strongly expressed (2.38 points). Hematoxylin-eosin. × 250, × 200

Table 1

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Intact control (n=8)</th>
<th>Control pathology (n=8)</th>
<th>Compound “Indolinoren”, 29.5 mg/kg (n=8)</th>
<th>Furosemide, 5 mg/kg (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis of nephrons</td>
<td>0</td>
<td>3.44±0.99*</td>
<td>2.05±0.29**</td>
<td>1.52±0.33**</td>
</tr>
<tr>
<td>Vacuolic dystrophy nephrotelium</td>
<td>0</td>
<td>2.19±0.13*</td>
<td>1.30±0.38**</td>
<td>1.11±0.32**</td>
</tr>
<tr>
<td>The presence of a cylinder in the lumen of the tubules</td>
<td>0</td>
<td>3.18±0.17*</td>
<td>1.83±0.39**</td>
<td>1.05±0.28**</td>
</tr>
</tbody>
</table>

**Notes:** deviations are probable in relation to intact control (p <0.05); **– deviations are probable relative to the control pathology (p <0.05); n – the number of animals in the group

6. Conclusions

1. According to the study of histostructure of kidneys on the glycerol acute renal failure model, the new compound propyl ester N-[(2-oxindolylidene-3)-2-oxacetyl] valine with the conditional name “Indolinoren” reduces the severity of necrosis of the nephrocytes of the tubules of the major parts of the nephrons and reduces the obstruction of the lumen of the tubules by homogeneous cylinders.

2. The new investigated compound “Indolinoren” on the severity of the protective effect on the histostructure of the renal kidneys of rats with glycerol acute renal failure syndrome is not inferior in compare to the diuretic furosemide.

3. The data obtained are the basis for further in-depth study of the compound “Indolinoren” as a perspective diuretic.

References

NEW POSSIBILITIES OF USE OF OAT RAW MATERIALS FOR INVESTIGATION OF BIOLOGICALLY ACTIVE COMPONENTS IN THERAPY OF EXPERIMENTAL DIABETES

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Метою роботи було запропонувати технологію аква динамічного екстрагування біологічно активних речовин із соломи вівса. Встановлено, що кількість екстракту та концентрація екстрагованих речовин залежать від тривалості процесу екстракції. Склад досліджуваного фітокомплексу дозволяє визначити склад фітосубстанцій.

Методи дослідження: для вивчення екстракту з соломи вівса його екстракцію здійснювали за допомогою ядерно-магнітного резонансу.

Результати: встановлено, що екстракт з соломи вівса містить значну кількість фітосубстанцій.

Ключові слова: Avena Sativa, солома, екстракція, поліфеноли, антиоксиданти, жирні кислоти, експериментальні тварини, цукровий діабет.