Matviychuk O. P., Matviychuk A. V., Taran A. V. Expermental research of the effects of benofilin on the functional renal function in the evalution of ethylene glycol toxic loss. Journal of Education, Health and Sport. 2019;9(2):493-499. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.2589444
http://ojs.ukw.edu.pl/index.php/johs/article/view/6673

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

Received: 05.02.2019. Revised: 11.01.2019. Accepted: 28.02.2019.

EXPERMENTAL RESEARCH OF THE EFFECTS OF BENOFILIN ON THE FUNCTIONAL RENAL FUNCTION IN THE EVALUATION OF ETHYLENE GLYCOL TOXIC LOSS

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Abstract

Effect of benofilin – a new compound among a new derivative of theophylline and hofitol (70 mg/kg) on functional renal function was studied based on the rat experimental model of acute kidney damage by ethylene glycol. The toxic products of the metabolism of ethylene glycol cause renal tubular damage resulting in acute renal failure (ARF). As a result of the application of benofilin its ability is established to prevent the death of animals in the first day of the experiment. By the ability to prevent impaired kidney function in rats benofilin exceeds the effect of the hofitol.

Keywords: benofilin, acute kidney damage, diuretic, nephroprotective activity, rats.

The prevalence and steady increase in the incidence of kidney disease is an important medical and social problem around the world. According to statistics on kidney disease and urinary tract disease, more than 10% of the world's adult population is affected, and this figure is constantly rising [8].

As a result of the progression of acute renal failure, the excretion of protein metabolism, the water-electrolyte balance, osmotic balance, and the role of the kidneys in maintaining stable blood pressure, erythropoesis, etc. [1, 4, 23].

Consequently, the search for effective and safe diuretics with nephroprotective activity remains one of the most relevant problems in the field of experimental pharmacology [5, 8, 18].

It is known that xanthine derivatives, inhibit the phosphodiesterase enzyme, increase the content of intracellular cAMP, are antagonists of adenosine receptors of cell membranes, participate in the regulation of many processes and exhibit a diverse pharmacological effect [19]. The anti-inflammatory effect of xanthine derivatives, the restoration of rheological properties of blood, and the effect on vascular tone are important links in preventing complications in renal pathology [4, 9].

Therefore, we have selected a new derivative of theophylline, 7-p-methylbenzyl-8-p-bromobenzylidenehydrazine-theophylline (conventional term "benofilin"), to investigate the protective effect on the kidneys studied in the experimental acute renal failure model.

This class of compounds was synthesized in the Zaporizhzhia State Medical University of the Ministry of Health of Ukraine under the direction of Professor Romanenko M.I.

The purpose of this paper the study of the protective effect of benofilin on functional renal function was investigated in an experimental model of acute kidney damage by ethylene glycol in rats.

Material & method

For the study of nephroprotective activity of the benofilin on the model of ethylene glycol formation of the kidneys of experimental animals, they were divided into four groups (for 6 rats in each): I – intact control (IC); II – control pathology (CP); III – pathology + benofilin in dose 35 mg⁄kg (ED_{50}); IV – pathology + hofitol in dose 70 mg⁄kg.

Benofilin and hofitol administered intragastrically in the solutions stabilized with a twin-80, in volume of 2 ml per animal. Animals from the CP group received an similar amount of water. Hofitol is chosen as a reference drug, since according to the instructions for medical use, a complex of biologically active substances that are part of it, has a diuretic, nephroprotective, antioxidant effect, improves metabolic processes in the body and reduces the urea content in the blood [7]. Ethylene glycol damage was induced by subcutaneous administration of ethylene glycol in the form of "chemically pure" in a dose of 6 ml/kg [13]. The test compound and the reference product were administered to the animals in the stomach in the prophylactic treatment for three days before the emergence of acute renal failure (ARF), at last for 40 minutes before the emergence of ARF, and 5 days during of ARF.

The efficacy of the new compound and the hofitol was determined by excretory function of the kidneys at the first day of experiment. Determined biochemical parameters of

urine and blood serum (content of urea, creatinine, uric acid, total protein, sodium ions, potassium etc.) [10].

An act on excretory function of the kidneys measured by diuretic value, glomerular filtration rate, concentration of creatinine in the plasma of the blood, homemade reabsorption, excretion of calcium and sodium ions, content of albumen and it excretion, filtration charge, reabsorption of sodium ions [3]. The performance indicators of kidney function were calculated according to the formulas which are described in the articles Yu V. Natochin [16] and O. T. Shyuk [22].

Determination of the concentration of creatinin in the urine was performed on the Folin method. Determination of the concentration of creatinin in the plasma was excised by photocolorimetric method [3].

The results were calculated using computer program data processing «Microsoft Excel 2010» and methods of variation statistics, $p \leq 0.05$ were taken as a probability criterion [6].

Results

During the study after 30 minutes after injection of ethylene glycol in animals of the CP group there was a macro hematuria. For the 3rd year observations were observed ataxia, areflexia, lateral position of animals, coma. During the first 12 hours all animals from the CP group were died (tab. 1).

Table 1

Mortality of rats in acute ethylene glycol injury (n=6)

Група	1 day	2 day	3 day	4 day	5 day
	died/ survive	died/ survive	died/ survive	died/ survive	died/ survive
Intact rats	0/6	0/6	0/6	0/6	0/6
Ethylene glycol nephropathy	6/6 (100%)	–	–	–	–
Ethylene glycol nephropathy +benofilin 35 mg/kg	2/4 (33%)*	3/3 (50%)*	3/3 (50%)*	3/3 (50%)*	3/3 (50%)*
Ethylene glycol nephropathy +hofitol 70 mg/kg	3/3 (50%)*	4/2 (67%)*	4/2 (67%)*	4/2 (67%)*	4/2 (67%)*

Note:

1. in the numeral - the number of dead animals
2. in the denominator - the total animals number in the group
3. in brackets % of those who died
4. * - probable differences with the control ($p<0.05$)

The mortality rate with the use of benofilin from the first 12 hours acute pathology is likely to decrease. The highest survival rate recorded at the end of the experiment (5 days). Improvement of animal survival proves the presence of nephroprotective properties in the test substance.

Oliguria stage of ACF was characterized decrease of diuresis more than 2 times through decrease glomerular filtration rate almost 5 times and reduction of tubal reabsorption by 1% compared to a similar indicator of intact rats.

The manifestation of pathology was accompanied by a significant reduction in the ability of the kidneys to excrete nitrogen-containing products of metabolism, which led to the occurrence of hyperazotemia and uremia. The level of creatinine and urea in blood serum of rats increased by 1.3 and 5.5 times, respectively. Urinary excretion significantly decreased by 1.5 and 2.3 times, respectively, relative to the animals in the intact control group. The degree of kidney damage indicated a decrease in urea clearance in 21.5 times in comparison with the control.

The progression of ethylene glycol arterial pressure indicates the development of proteinuria which is characterized by a much greater loss of protein in the urine (by 3.2 times) at the same time a significant decrease in serum compared with the level of intact animals.

The use of benofilin contributed to the reliable normalization of the impaired functional state of the kidneys in all of the above-mentioned indices, except for the level of protein in the urine, which tended to decrease.

Significant restoration of diuresis with the use of benofilin on the background of ethylene glycol nephropathy was characterized by its increase in 2.3 times compared with the level in the group of pathology and even 10.7% in relation to the level of inflammation. However, for this indicator, benofilin is insignificant (8%) inferior to the reference product of hofitol.

This dynamics of diuresis against the background of the benofilin is associated with a 5.4-fold increase in GFR and a significant normalization of tubal reabsorption, which is only 0.14% below the norm in comparison with ethylene glycolic arterial pressure.

The indicated indicators indicate the normalizing effect of the benofiline on the nitrogen function of the kidneys. Hypoazothemic activity of the benofilin is characterized by a significant decrease in the concentration of creatinine and serum urea in 1,2 and 6,5 times with a simultaneous increase in their excretion in the urine in 1,4 and 1,9 times, respectively, in comparison with the CP group.

The informative parameter in the experimental conditions was the clearance of urea, which was reliably normalized against the background of the benofilin. The indicator was slightly lower than that in the IR group, but compared to ethylene glycol, the ARF increased 18-fold.

The total hypoazothemic activity of the benofilin in all studied parameters also exceeds the similar effect of hofitol: an average of 4-12% on creatinine content, in the dynamics of changes in urea level - in 1,5-2,6 times, and in urea clearance - 4 times comparatively with a reference preparation.

An important criterion for the nephroprotective effect of benofilin can also be considered its ability to prevent protein loss from the urine as one of the principal signs of renal insufficiency. Benofilin normalized the content of total serum protein to almost the level of IR (66.4 ± 1.10 g / l) ($P \leq 0.05$). At the same time, in animals from the hofitol administration group, there was only a slight tendency to increase serum protein levels, which remained at almost CP level (57.8 ± 1.80 g / l). The decrease in protein content in animal urine under the influence of benofilin and hofitol had a tendency of 26.1% and 8.7% respectively.

Conclusion

Thus, based on the results of an experimental analysis of the influence of the benofilin on the parameters of the functional state of kidneys in rats in the experiment, that the test substance on the background of acute toxic damage to the kidneys with ethylene glycol reliably normalizes all investigated kidney parameters and showed a higher nephroprotective effect than the reference medicine hofitol.

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