Research Article

Specific and Acute Toxicity Estimate of New Domestic Nootropic Preparation

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Abstract

Purpose of the Research: To comparative specific properties and acute toxicity researching of a new domestic no otropic "Nootrotem" preparation. **Materials & Methods:** The research object was "Nootrotem" preparation; it is solution for infusion produced by LLC "Temur Med Farm" Uzbekistan and comparison preparations "Mexidol" and "Piracetam". The preparation anti hypoxic activity was studied under model of normobaric hypoxic hypoxia in experiments on 18 white male mice, divided into 3 groups. They are Group 1 (control)-0.9%, 0.3 ml volume NaCl solution were injected intravenously during 3 days; Group 2 (experimental)-within 3 days no otrotem was injected at the dose of 337.5 mg/kg (0.3 ml); Group 3 (comparison)-for 3 days, diluted comparison preparations "Mexidol" and "Piracetam" were injected intravenously at a dose of 337.5 mg/kg (0.3 ml). Acute toxicity was investigated by using a conventional method on white outbred male mice for heads. The research experiments were done in two stages. In the first stage, no otrotem was injected once into the tail vein of white mice as follows: Group 1 (6 mice)-intravenous infusion at a dose of 112.5 mg/kg (0.1 ml); Group 2 (6 mice)-intravenous infusion at a dose of 337.5 mg/kg (0.3 ml); Group 3 (6 mice)-intravenous infusion at a dose of 562.5 mg/kg (0.5 ml). In the second stage experiment solution identical to the No otrotem preparation (Mexidol 1 ampoule (5 ml)+Piracetam 1 ampoule (5 ml)+0.9% NaCl, (90 ml) was prepared from the preparations "Mexidol" and "Piracetam", was administered once into the tail vein of white mice as follows: Group 1 (6 mice)-intravenous infusion at a dose of 112.5 mg/kg (0.1 ml); Group 2 (6 mice)-intravenous infusion at a dose of 337.5 mg/kg (0.3 ml); Group 3 (6 mice)-intravenous infusion at a dose of 562.5 mg/kg (0.5 ml). After the completion of the experiment, the LD50 and the toxicity class of the preparations are determined. The data obtained were statistically processed using the STATISTICA program. **Results:** "Nootrotem" preparation on the model of normobaric hypoxic hypoxia increases the Resistance of mice to hypoxia: At a dose of 337.5 mg/kg it significantly lengthens the life span of animals by 23.6%, making 11.4 ± 0.43 minutes, while as in the control group of mice, this indicator was 9.3 ± 0.45 min. LD50 of Nootrotem and Mexidol+Piracetam solution was >562.5 mg/kg. **Conclusion:** Preparations have an equally reliable anti hypoxic effect and a low toxicological characteristic.

Keywords: Nootrotem; Piracetam; Mexidol; Normobaric hypoxia; Acute toxicity

Introduction

In recent years, much attention has been paid to providing the population with domestically produced pharmaceuticals, the release of new import-substituting drugs, affordable drugs from an economic point of view; important tasks have been identified for "the development of the pharmaceutical industry, as well as improving the provision of the population and medical institutions with cheap, high-quality drugs". [1-10] In particular, to meet the needs of the population with pharmaceutical products by creating new drugs from local raw materials, which are not inferior in activity to foreign analogues. [11,12] In this regard, the search for new drugs is being carried out, their introduction into the pharmaceutical industry. Active work is being carried out by various pharmaceutical joint-stock companies to develop new drugs, in particular drugs with nootropic action. The need for these drugs among the population is very high due to the high frequency of various forms of encephalopathy and anxiety-depressive states, stress, as well as lengthening the life expectancy of the population. [13-18] With the advent of nootropics, it became possible for the first time to have a targeted pharmacological effect on cognitive functions, manifestations of mental and neurological deficits that form in organic brain damage. The main characteristic of nootropics is an activating specific effect on the higher integrative functions of the brain

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and restoration of disorders of higher nervous activity. [5,7] Thus, nootropic drugs lead to an improvement in memory, attention, thinking, orientation, expansion of the volume of perception, an increase in the ability to analyze and assess the situation, and make decisions. Basically, these drugs are imported imported drugs, they are expensive and inaccessible for low-income families. This dictates the need for the development and production of nootropic drugs in the republic, mainly from local raw materials, the study of molecular mechanisms of positive action, toxicological characteristics of new drugs for introduction into the pharmaceutical industry of the republic. One of these drugs is Nootrotem (solution for infusion), created by Temur Med Farm LLC, Uzbekistan. [14]

Purpose of the research

Purpose of the study: Comparative study of the specific properties and acute toxicity of a new domestic nootropic drug "Nootrotem".

Materials and Methods

The object of the study was the drug "Nootrotem" a solution for infusion (s. 0010219, s.g. 02/2021) produced by LLC "Temur Med Farm" Uzbekistan. The active ingredients in Nootrotem are Methylethyl Hydroxyl Pyridine Succinate (MEHPS) and Piracetam. In this regard, as a comparison, we used preparations: "Mexidol" a solution for intravenous and intramuscular administration of 50 mg/ml, 5 ml each (p. 020418, 05/2021 No. and registration date DV/X 00578/07/15 17/07/15 B-250-9527110 RUz 28/07/10) manufactured by FKP "Armavir Biofabrika" (Russia) and "Piracetam"-solution for injection 200 mg/ml (p. 1370718, 08 / 2023 No. and date of registration DV/X 01562/04/16 01/04/16 B-250-95 11801RUz 12/05/06), manufactured by LLC Borisov plant of medical products (Belarus).

The antihypoxic activity of the preparations was studied on a model of normobaric hypoxic hypoxia in experiments on 18 white male mice weighing 20 g-23 g. [10] For the experiment, the mice were divided into 3 groups of 6 heads: Group 1 (control)-for 3 days, 0.9% NaCl solution was injected intravenously in a volume of 0.3 ml; Group 2 (experimental)-for 3 days Nootrotem was injected intravenously at a dose of 337.5 mg/kg (0.3 ml); Group 3 (comparison)-diluted comparison drugs were injected intravenously for 3 days "Mexidol" and "Piracetam", produced by Borisov et al. plant of medical preparations, Belarus at a dose of 337.5 mg/kg (0.3 ml). On the 3rd day of the experiment, 30 minutes after the last injection of the drug, 2 mice were placed in sealed containers with a volume of 250 ml. The time from the moment of placing in the dish to the cessation of breathing and death of the animal was recorded. The results obtained were expressed as a percentage of the values of the control group which was taken as 100%.

Acute toxicity was studied by the generally accepted method described in the literature, a single administration of the drug with the definition of the toxicity class. [3,9] For the experiments, 36 white outbred male mice were used, weighing 19 g-21 g, kept in quarantine for 14 days. The experiments were carried out in two series. In the first series, Nootrotem was injected once into the tail vein of white mice as follows: Group 1 (6 mice)-at a dose of 112.5 mg/kg (0.1 ml); 2nd group (6 mice)-at a dose of 337.5 mg/kg (0.3 ml); Group 3 (6 mice)-at a dose of 562.5 mg/kg (0.5 ml). In the second series of the experiment, a solution identical to the Nootrotem preparation (Mexidol 1 ampoule (5 ml)+Piracetam 1 ampoule (5 ml+90 ml 0.9% NaCl) was prepared from the preparations "Mexidol" and "Piracetam" and was injected once into the tail vein of white mice as follows: Group 1 (6 mice)-iv at a dose of 112.5 mg/kg (0.1 ml); Group 2 (6 mice)-at a dose of 337.5 mg/kg (0.3 ml); Group 3 (6 mice)-at a dose of 562.5 mg/kg (0.5 ml). On the first day of the experiment, the animals were observed hourly, then every day for 2 weeks in a vivarium, the animals of all groups were monitored for general condition and activity, behavioral features, reaction to tactile, pain, sound and light stimuli, frequency and the depth of respiratory movements, the rhythm of heart contractions, the condition of the hair and skin, the position of the tail, the amount and consistency of fecal masses, the frequency of urination, changes in body weight, and other indicators. All experimental animals were kept in the same conditions and on a common diet with free access to water and food. After the completion of the experiment, the LD50 and the toxicity class of the drug are determined. The data obtained were statistically processed using the STATISTICA program according to the paired Student's t test.

Results and Discussion

The obtained results in the research of the antihypoxic effect showed that the drug Nootrotem on the model of normobaric hypoxic hypoxia increases the resistance of mice to hypoxia: At a dose of 337.5 mg/kg it significantly lengthens the life span of animals by 23.6%, making 11.4 min ± 0.43 min, while in the control group of mice this indicator was 9.3 min ± 0.45 min [Table 1].

Under similar conditions, reference drugs "Mexidol" and "Piracetam" at a dose of 337.5 mg/kg also showed an antihypoxic effect, extending the life expectancy of animals by 23.4%. We did not reveal any differences in the life expectancy

Table 1: Antihypoxic effect of the drug "Nootrotem", "Mexidol®" and "Piracetam" on the model of normobaric hypoxia, M ± m, n=6.

Weight, g	Dose, mg/kg	Solution volume, ml	Life span, min	% effect
Control group, 0,9% NaCl				
21,5 ± 1,05		0,3 мл	9,2 ± 0,76	-
"Nootrotem", produced by LLC "Temur Med Farm" Uzbekistan				
21,3 ± 1,2	337,5	0,3 мл	11,4 ± 0,43, P<0,05	23,6
Mexidol®+Piracetam				
21,67 ± 1,2	337,5	0,3 ml	11,35 ± 0,3, P<0,05	23,4

Table 2: Determination of acute toxicity "Nootrotem" and comparison drugs "Mexidol®" + "Piracetam", M ± m, n=6.

Groups	Nootrotem				Mexidol®+Piracetam			
	Volume		Route of administration	Results	Volume		Route of administration	Results
	mg/kg	ml			mg/kg	ml		
1	112,5	0,1	i / v	0/6	112,5	0,1	i / v	0/6
2	337,5	0,3	i / v	0/6	337,5	0,3	i / v	0/6
3	562,5	0,5	i / v	0/6	562,5	0,5	i / v	0/6
LD_{50}					>562,5 mg/kg			

of experimental animals in the experimental group and the comparison group.

Therefore, the study drug "Nootrotem" solution for infusion (p.0010219, s.y. 02/2021), produced by LLC "Temur Med Farm" (Uzbekistan) in comparison with drugs "Meksidol" solution for intravenous and intramuscular administration of 50 mg/ml, 5 ml each (p. 020418, 05/2021 No. and date of registration DV/X 00578/07/15 17/07/15 B-250-9527110 RUz28/07/10), manufactured by FKP "Armavir Biofactory"(Russia) and "Piracetam"-solution for injection 200 mg/ml (p. 1370718, 08/2023 No. and date of registration DV/X 01562/04/16 01/04/16 B-250-95 11801 RUz 12/05/06), produced by LLC Borisov plant of medical preparations (Belarus) have an equivalent reliable anti hypoxic effect.

During to study the acute toxicity of drugs, the following data were obtained [Table 2]. Group 1 (dose 112.5 mg/kg): after administration of the drug during the day, the mice remained active; no changes in behavior and functional state were observed. The condition of the coat and skin was normal without changes, they did not refuse food and water, and the death of mice was not observed. On the second day and in the subsequent period of observation, no pathological changes in the behavior and physiological parameters of the mice were found. Water and feed intake was normal, growth and development were not observed. There was no death of mice within 14 days.

Group 2 (dose 337.5 mg/kg), after administration of the drug during the day, mice were active, no visible changes were observed in behavior and functional state. The condition of the coat and skin was normal without changes, they did not refuse food and water, and the death of mice was not observed. On the second day and in the subsequent period of observation, there were no pathological changes in the behavior and physiological parameters of the mice. Water and feed intake was normal, growth and development were not observed. There was no death of mice within 14 days.

Group 3 (at dose 562.5 mg/kg), after administration in mice showed short-term lethargy and inactivity which disappeared after 30 minutes-40 minutes. After 1 hour, the mice returned to their previous state, their behavior was active, and the physical parameters did not deviate from the norm. On the second day and during the entire observation period for 14 days, no changes in behavior and other physical parameters were observed in mice, mice willingly consumed food and water, reactions to light and sound stimuli remained normal, hair and skin were clean, urination and fecal excretion normally, the weight and growth of the mice did not lag behind in development. Mice death was not observed.

Since, according to the literature, the volume of injected fluid with a single intravenous administration is no more than 0.5 ml, the introduction of a larger dose of the drug was not possible. The LD50 of the Nootrotem drug and the Mexidol+Piracetam solution was >562.5 mg/kg.

Conclusion

The main component of Nootrotem MEGPS improves microcirculation and rheological properties of blood, reduces platelet aggregation, which leads to improved blood supply to the brain and metabolism in nerve cells. [4] Proved antihypoxic, membrane-protective, nootropic, anticonvulsant, anxiolytic action, increases the body's resistance to stress. [18] Established hypolipidemic, hypocholesterolemic properties. [15] MEHPS inhibits lipid peroxidation processes, increases the activity of superoxide dismutase, [19] modulates the activity of membrane bound enzymes (calcium-independent phosphodiesterase, adenylate cyclase, acetylcholinesterase), receptor complexes (benzodiazepine, GABA, and through HIF [20] (hypoxia-inducible factor), which enhances tissue peroxidation, increasing tubular permeability for ions (Na+, K+, Ca++) and thus creates favorable conditions for enhancing the membrane protective properties of the cell, and helps to maintain the structural and functional organizing bio membranes, transporting neurotransmitters and improving synaptic transmission, increases the content of dopamine in the brain. [2,6] It causes an increase in the compensatory activity of aerobic glycolysis and a decrease in the degree of inhibition of oxidative processes in the Krebs cycle under conditions of hypoxia, with an increase in the content of ATP, creatine phosphate and activation of the energy-synthesizing functions of mitochondria, stabilization of cell membranes. [6] MEHPS normalizes metabolic processes in the ischemic myocardium, reduces the zone of necrosis, restores and improves electrical activity and myocardial contractility, and also increases coronary blood flow in the ischemic zone, and reduces the consequences of reperfusion syndrome in acute coronary insufficiency. [8]

Another component of Nootrotem, Piracetam, is also a nootropic agent, which directly affects the brain, improving cognitive properties and mental performance. [17] The mechanism of its action is associated with a change in the rate of propagation of excitation in the brain, improvement of metabolic processes in nerve cells, microcirculation and rheological characteristics of blood, without causing vasodilation. Piracetam inhibits platelet aggregation and restores the elasticity of the erythrocyte membrane, reduces their adhesion, reduces the level of fibrinogen and willi brand factor. [1]

All achieved data based on the following conclusions

1. The drug Nootrotem on the model of acute normobaric hypoxia increases the survival rate of animals which indicates its antihypoxic effect.
2. The LD50 of the Nootrotem drug and the Mexidol+Piracetam solution were >562.5 mg/kg which indicates the absence of an acute toxic effect of the drugs.

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