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Elimination profile of the new antihypoxant OKAGERM-4 under normal conditions and confined space hypoxia model

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Key words: pharmacokinetics, elimination, OKAGERM-4, confined space hypoxia

The problem of pharmacological protection of the body from complications of hypoxic conditions of various origins is relevant and requires immediate solution. The most vulnerable to exogenous hypoxia are people engaged in extreme types of occupational activities (miners, divers, submariners, pilots, firefighters, rescuers, etc.), in the conditions of abnormal operation of the oxygen supply system in confined spaces. This form of acute oxygen deficiency is better known as confined space hypoxia (CSH) [1–3].

The drugs with antihypoxic effects available to general practitioners do not fully meet the requirements and demands of practical medicine due to low therapeutic and prophylactic efficacy, small breadth of therapeutic action and numerous reactions [4].

Thus, the need to find and develop highly effective and safe antihypoxic drugs is not in doubt and has long been the subject of research for scientists in various fields, and also is a particularly important task of pharmacological science.

Today, in terms of the search for new drugs with high antihypoxic potential, the most promising are heterometallic complexes of germanium and 3d-metals (zinc, copper, manganese) with citric and tartaric acids, which are notable for a variety of pharmacodynamic effects, acceptable pharmacokinetic profile and relative harmlessness [5].

Previously performed toxicometric [6] and screening studies [7] of potential antihypoxants in a number of bimetallic complexes of germanium (IV) with anions of citric and tartaric acids under CSH model experimentally proved high antihypoxic activity and harmlessness of the coordination compound of germanium with manganese based on tartaric acid [manganese (II) tartrate germinate (IV)] under the laboratory code OKAGERM-4, which was first synthesized in the laboratory of the Department of General Chemistry and Polymers of Odessa Mechnikov National University under the leadership of prof. I. Y. Seifullina.

The aim of the study – to carry out comparative pharmacokinetic analysis of OKAGERM-4 at the stage of its elimination from the central chamber under normal conditions and CSH model.

Materials and methods. The experiments were performed on 48 white nonlinear rats of both sexes weighing 170–200 g after quarantine. The animals received a standard diet in the form of granular feed according to established norms and free access to water. The research was conducted in the SI «Institute of Pharmacology and Toxicology of the National Academy of Medical Sciences of Ukraine» in

accordance with the guidelines of the State Enterprise «State Expert Center of the Ministry of Health of Ukraine». The study protocol complies with the bioethical standards and provisions of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), as well as the Law of Ukraine No. 3447–IV «On Protection of Animals from Cruel Treatment» of February 21, 2006.

Experimental animals were randomly divided into two groups: animals without pathology (normal) and experimental animals (CSH). The experimental model was a pathological process that develops in animals in confined spaces. CSH simulations were performed by placing rats for 25 min in glass sealed containers (10 cm^3), which were turned upside down and immersed in a tray with water, which was used as a hydraulic lock.

OKAGERM-4 was injected to the rats of both groups once intraperitoneally according to the dosage regimen previously developed by us in pharmacometric studies [8]: 96.8 mg/kg as a 1 % aqueous solution 40 min before the start of CSH simulation. Blood sampling of rats was carried out in the dynamics: 45 min, 3 h, 6 h and 24 h after OKAGERM-4, the content of which in the blood was determined using a validated method by the amount of germanium in the biological material [9] on a spectrophotometer Smart-Spec™ Plus Spectrophotometer (Bio – Rad Laboratories, Inc, USA).

The following pharmacokinetic parameters characterizing the process of OKAGERM-4 elimination from the central chamber were determined: half-life ($t_{1/2\beta}$, h), elimination rate constant ($К_{10}$, h^{-1}), mean residence time (MRT, h) and total clearance (Cl_t, mL/h/kg); the parameters were calculated by the computer program Phoenix WinNonLin 8.1 (Pharsight Corp., Certara L.P, USA) [10] using a two-compartment model [11].

Statistical processing of the data obtained was performed using the program StatSoft Statistica 10. Before applying the statistical criteria, the hypothesis of the normal law of distribution of random variables according to the Shapiro – Wilk criterion (significance level 0.01) was tested. Since all the above parameters are subject to the normal distribution law, Student's t-test (significance level 0.05) was used for comparison.

Results and their discussion. Calculations of a number of pharmacokinetic parameters characterizing the intensity of the process of OKAGERM–4 elimination from the body of rats without pathology and with the modeled form of hypoxia were performed on the basis of pharmacokinetic curves obtained in semi–logarithmic coordinates, presented in Figure. For the most part, the pharmacokinetic curves obtained are presented in the form of a straight line depicting the linear dependence of the elimination process of studied antihypoxant under normal conditions and in CSH.

The comparative analysis of the calculated pharmacokinetic parameters of OKAGEM-4 elimination from the central chamber under normal conditions and CSH (Table) shows that the rate of the studied process remains almost the same, which is actually confirmed by the values of the rate constants.

Thus, the acute form of modeled hypoxic syndrome (CSH) does not have a significant effect on the rate of elimination processes, which directly depend on the intensity of biotransformation and excretion of potential antihypoxant.

The assessed half-life values in the groups of «normal» animals and those

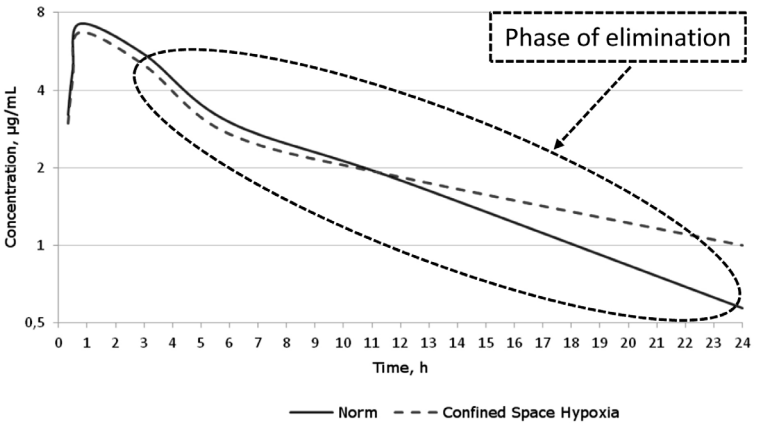

Figure. Pharmacokinetics of OKAGERM-4 in semi-logarithmic coordinates in the normal blood and under the conditions of CSH after single intraperitoneal injection to rats at a dose of 96,8 mg/kg

who underwent hypoxia were very close and amounted to 11,33 and 11,83 h, respectively. This state of affairs was highly expected, since the values of $t_{1/2\beta}$ and $К_{10}$ have an inverse and close mathematical interdependence. There were also no significant effects of CSH on the nature of clearance in both study groups of animals, the values of which were – 1,37 mL/h/kg (norm) and 1,42 mL/h/kg (CSH). In addition, similar values of the mean residence time (7.30 and 7.36 h) of OKAGERM-4 were identified in the studied groups of animals.

It should be noted that according to all pharmacokinetic parameters that characterize the elimination process of OKAGERM-4 from the central chamber ($t_{1/2\beta}$, $К_{10}$, Cl_t, MRT) there is no significant difference ($P > 0.05$) between the groups of «normal» animals and those exposed to acute hypoxic hypoxia with progressive hypercapnia.

In order to interpret the data obtained in the experiment as correctly as possible, we find it necessary to consider the functional state of those organs that participate in the xenobiotics elimination processes, i. e. liver and kidneys under the conditions of modeled pathology of hypoxic origin.

It is not possible to determine the exact time of the beginning of the elimination phase, because the absorption and elimination of the antihypoxant under study occur simultaneously for some period. At the same time, the analysis of OKAGERM-4 pharmacokinetic curves in semi-logarithmic coordinates (Figure)

Table

Pharmacokinetic parameters that characterize the process of OKAGERM-4 elimination from the central chamber under normal conditions and CSH after single intraperitoneal injection to rats at a dose of 96.8 mg/kg (M ± m, n = 6)

Parameter	Symbol, units of measurement	Group of animals		P*
		Norm	CSH	
Elimination rate constant	K_{10}, h^{-1}	0.26 ± 0.05	0.23 ± 0.03	> 0.05
Half-life	$t_{1/2\beta}$, h	11.33 ± 4.90	11.83 ± 3.16	> 0.05
Total clearance	Cl_t, mL/h/kg	1.37 ± 0.28	1.42 ± 0.18	> 0,05
Mean residence time	MRT, h	7.30 ± 0.89	7.36 ± 0.55	> 0.05

*Note. *In comparison with healthy animals (norm).*

shows that at the 3 h point the curves «refract» and become maximally linear; this is where, in our opinion, the elimination phase actually begins. It should be noted that based on the data of comparative analysis of pharmacokinetic curves and the course of clinical symptoms of CSH, the process of elimination of OKARERM-4 coincides with the beginning of the posthypoxic period in animals exposed to acute hypoxia.

As a matter of discussion, it is worth noting the well–known fact that the indicators that characterize the process of elimination, as well as distribution, largely depend on the state of the macro– and microcirculation system. It is shown that in animals exposed to hypoxia in the early posthypoxic period there is a very rapid stabilization of vital functions, blood circulation and microcirculation, which can have a positive effect on elimination processes under the conditions of the experiment under study.

Given the results of the works performed in the laboratory of the Department of Pharmacology of Lugansk State Medical University, studying the coordination organogermanium compounds with bioligands in the treatment of acute oxygen deficiency (hypoxic hypoxia, traumatic rhabdomyolysis, traumatic brain injury), using EPR–13 spectrometry [12–14], it is possible to assume that OKAGERM-4 has the ability to induce the activity of cytochrome P-450, which provides protection of biotransformation processes under the conditions of CSH, which in a sense affects the elimination in general.

Also, based on the results of studying the distribution of potential antihypoxant from the central chamber to the kidneys [15], namely the tropism to renal tissue, as a hypothesis we assume that OKAGERM-4 has a nephroprotective effect in the modeled form of hypoxic syndrome and enhances diuresis.

Conclusions

According to the results of complex comparative pharmacokinetic studies of the elimination process of OKAGERM-4 from the central chamber for all the studied parameters ($t_{1/2\beta}$, $К_{10}$, Cl_t, MRT), the absence of a probable ($P > 0.05$) difference between the group of «normal» animals and those affected by the CSH was established. This indicates the stability of the coordination compound of germanium with manganese and tartaric acid in relation to biotransformation and excretory processes both directly under the influence of acute hypoxic hypoxia with progressive hypercapnia and in the early posthypoxic period.

Thus, the generalization of the results obtained as to this stage of pharmacokinetics allows us to note that regardless of the presence of hypoxic damage in the body, the elimination process of OKAGERM-4 from the central chamber is approximately the same.

In conclusion, it should be noted that the rational use of a potential antihypoxant in clinical conditions will not require physicians to adjust the dosage regimen during the treatment of patients subjected to CSH.

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Профіль елімінації нового антигіпоксанта ОКАГЕРМ-4 у нормі та на моделі гіпоксії замкненого простору

Проблема фармакологічного захисту організму від ускладнень гострої гіпоксії, що розвивається в закритому просторі, гіпоксії замкненого простору (ГЗП), є актуальною й потребує невідкладного вирішення. Сьогодні в плані пошуку нових лікарських засобів з великим антигіпоксичним потенціалом найперспективнішими є гетерометалічні комплекси германію та 3d-металів (цинк, мідь, манган) з лимонною та винною кислотами. Токсикометричними та скринінговими дослідженнями доведено високу антигіпоксичну активність і нешкідливість координаційної сполуки германію – манган (II) тартратогерманат (IV) за лабораторним шифром ОКАГЕРМ-4.

Мета дослідження – здійснити порівняльний фармакокінетичний аналіз ОКАГЕРМ-4 на етапі його елімінації з центральної камери в нормі та на моделі ГЗП.

Досліди виконані на 48 нелінійних білих щурах обох статей масою 170–200 г, розподілених на дві групи: контрольну та дослідну (щури, в яких моделювали ГЗП шляхом розміщення їх на 25 хв в ізольованих гермооб'ємах 10 $см^3$). Для визначення фармакокінетичного профілю встановлювали рівень ОКАГЕРМ-4 за вмістом германію колориметричним методом у сироватці крові тварин через 45 хв, 3 год, 6 год і 24 год після одноразового внутрішньоочеревинного введення в дозі 96,8 мг/кг.

На підставі отриманих напівлогарифмічних кривих безпосередньо було виконано розрахунок низки фармакокінетичних параметрів, які характеризують процеси елімінації ОКАГЕРМ-4 із центральної камери: $t_{1/2\beta}$ (норма) 0,26 год ± 0,05 год; $t_{1/2\beta}$ (ГЗП) 0,23 год ± 0,03 год; K_{10} (норма)

11,33 год$^{-1}$ ± 4,90 год$^{-1}$; K_{10} (ГЗП) 11,83 год$^{-1}$ ± 3,16 год$^{-1}$; MRT (норма) 1,37 год ± 0,28 год; MRT (ГЗП) 1,42 год ± 0,18 год; Cl_t (норма) 7,30 мл/год/кг ± 0,89 мл/год/кг; Cl_t (ГЗП) 7,36 мл/год/кг ± 0,55 мл/год/кг.

Таким чином, експериментально встановлено, що процес елімінації ОКАГЕРМ-4 з центральної камери відбувається приблизно однаково незалежно від наявності в організмі гіпоксичного пошкодження.

Ключові слова: фармакокінетика, елімінація, ОКАГЕРМ-4, гіпоксія замкненого простору

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The problem of pharmacological protection of the body from complications of acute hypoxia, which develops in a confined unventilated space, confined space hypoxia (CSH), is relevant and requires immediate solution. Today, in terms of finding new drugs with high antihypoxic potential, the most promising are heterometallic complexes of germanium and 3d-metals (zinc, copper, manganese) with citric and tartaric acids. Toxicometric and screening studies proved high antihypoxic activity and harmlessness of the germanium – manganese (II) tartrate germanate (IV) coordination compound under the laboratory code OKAGERM-4.

The aim of the study – to carry out comparative pharmacokinetic analysis of OKAGERM-4 at the stage of its elimination from the central chamber under normal conditions and in the modeled CSH.

The experiments were performed on 48 nonlinear white rats of both sexes weighing 170–200 g, divided into two groups: control and experimental (rats in which CSH was modeled by placing them for 25 min in hermetic 10 cm^3 sealed containers). In order to determine the pharmacokinetic profile, the level of OKAGERM-4 was determined by germanium content using the colorimetric method in blood serum 45 min, 3 h, 6 h and 24 h after a single intraperitoneal injection to rats at a dose of 96.8 mg/kg.

Based on the semi-logarithmic curves obtained, a number of pharmacokinetic parameters were directly calculated, those that characterize the processes of OKAGERM-4 elimination from the central chamber: $t_{1/2\beta}$ (norm) 0.26 h ± 0.05 h; $t_{1/2\beta}$ (CSH) 0.23 h ± 0.03 h; K_{10} (norm) 11.33 h^{-1} ± 4.90 h^{-1}; K_{10} (CSH) 11.83 h^{-1} ± 3.16 h^{-1}; MRT (norm) 1.37 h ± 0.28 h; MRT (CSH) 1.42 h ± 0.18 h; Cl_t (normal) 7.30 mL/h/kg ± 0.89 mL/h/kg; Cl_t (CSH) 7.36 mL/h/kg ± 0.55 mL/h/kg.

Thus, it has been experimentally established that the process of OKAGERM-4 elimination from the central chamber occurs approximately the same way regardless of the presence of hypoxic damage to the body.

Key words: pharmacokinetics, elimination, OKAGERM-4, confined space hypoxia

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