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DEVELOPMENT OF A PHARMACETICAL ANTICANCER GEL BASED ON DOXORUBICIN AND SILICONE NANOTECHNOLOGY

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РАЗРАБОТКА ФАРМАЦЕВТИЧЕСКОГО ПРОТИВООПУХОЛЕВОГО ГЕЛЯ С НИОСОМАЛЬНЫМ ДОКСОРУБИЦИНОМ

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Developed a gel based on pharmacological niosomes silicone nature incorporated with the anticancer drug – doxorubicin at 5 mg/ml to enhance the efficacy of cancer treatment. Using prototype gel doxorubicin niosomes composition provides greater bioavailability of the antibiotic and significantly prolongs its retention time in blood concentrations. Even 48 hours after gel application prototype drug concentration in the blood exceeds control values three times. The transdermal route of administration of doxorubicin can reduce the cardiotoxic effect of the drug, dyspeptic reactions, reduce the likelihood of necrosis at the injection site, which significantly extends the use of the drug among patients with heart failure, diseases of the gastrointestinal tract.

Key words: doxorubicin, niosomes, niosomal gel, skin cancer

Разработан фармакологический гель на основе ниосом кремнийорганической природы с включенным в них противоопухолевым препаратом – доксорубицином в концентрации 5 мг/мл для повышения эффективности лечения раковых заболеваний кожи и снижения кардиотоксичности и гепатотоксичности. Изучение фармакокинетики полученного геля с доксирубицином показало пролонгированный эффект применяемого лекарственного средства за счет постепенного высвобождения препарата, что позволило поддерживать высокую концентрацию антибиотика в крови длительное время.

Ключевые слова: доксорубицин, ниосомы, ниосомальный гель, рак кожи

One highly effective anticancer drug is doxorubicin – an antibiotic with a cytostatic effect. This drug is more than 30 years old and is used as a treatment for tumors of different organs [2, 4, 12]. Doxorubicin is mainly metabolized by the liver following intravenous administration which dissipates rapidly from the blood stream and is accumulated in the parenchymal organs. The disadvantages of the drug are that it is toxic for the heart and liver, which limits its use for the patients with tumor diseases [8, 10, 11]. To broaden the range of possible applications of the drug by reducing its toxicity and by not allowing the use of doxorubicin in free form and in the form of drug composing of doxorubicin and carrier (transport system) drugs which affects its biodistribution in the body, in particular, reducing the possibility of entering the heart and liver which can be successful. Niosomal doxorubicin, a dosage form in which the antibiotic is enclosed in a microcapsules silicone h the (niosomes), which consist of PEG-12 dimethicone [3, 5]. With the method used for encapsulation monolayer capsules have a size of less than 100 nanometers, allowing the delivery of the drug to prolong its effect due to the gradual release, reducing the toxicity of doxorubicin and by reducing the amount of side effects on the body [7, 9]. Furthermore, there is the possibility of creating a new niosomal form of medication for transdermal application of the drug that can be effectively used for the treatment of skin cancer.

The purpose of our study was the experimental proof of the benefits using the niosomal form of doxorubicin and the creation of prototype drug for transdermal application.

Material and Methods. For forming niosomes as a surface active compound using PEG-12 dimethicone. For the preparation of the drug, 500 mg of doxorubicin (dose of 5 mg in 1 ml in the finished product) was dissolved by stirring in 90 ml of water in preparation for injection. An additional 10 ml of PEG-12 dimethicone was added to this solution. The formation of vesicles occurred after 5 minutes in a homogenizer at room temperature Administration of doxorubicin in the niosomes is by ultrasonic methods. Dispersion of niosomes and the drug is achieved by placement in a bioruptor.

Parameters ultrasonic frequency – 20 kHz, power – 200 Watts, exposure time – 10 minutes. Thus the unilamellar niosomes that are formed are smaller than 100 Nm by the inclusion of doxorubicin at over 58%. An emulsion is then created with emulsified niosomes in 1000 ml of purified water, containing 50 ml of a gelling agent. The final stage is to form a «spatial grid» polymer by the addition of 20 ml of triethanolamine [1].

The next step is to study the pharmacokinetics of doxorubicin encapsulated in silicone niosomes. To determine the pharmacokinetics of doxorubicin encapsulated in a transdermal gel that was used in both an experimental and a control group of animals. Before the start of the experiment the animals were given food. Two hours later, the animals were weighed and distributed into groups. Selection was carried out in the groups arbitrarily using as a criterion their body weight. Individual body weights did not deviate from the value in the group by more than 10%. The experimental and control group consisted of 17 male of chinchilla rabbits weighing between 5000–5500 g. The experimental group included a niosomal applied doxorubicin gel; the control group received a gel consisting of free doxorubicin. Experimental and control groups received an initial dose of 20 mg/m^2 doxorubicin. The gel was applied to an epilate skin surface 5x5 cm abdominally. Throughout the experiment at intervals of (6 hours, 12 hours, 24 hours and 48 hours) the venous blood of the rabbits were taken (from the ear vein) by an amount of 0.3 ml to determine the concentration of doxorubicin. A serum was prepared from the blood by centrifugation. The antibiotic concentration in the samples was determined by reversed-phase high-performance liquid chromatography (RP-HPLC) with fluorometric detection using chromatograph UltiMate – 3000 (Dionex, USA). For this purpose, column ReproSil Pur C18 – AQ, and pre-column Teknokroma Novafix C18 were used. The mobile phase used was acetonitrile and a solution of sodium hydrogen phosphate (Na_2HPO_4) and triethylamine (0.05 vol %). The pH was adjusted to 4,6 by using a citric acid solution. Optimal elution conditions are reached at the rate of 0,50 ml/min for 10 min and a column temperature of 35C. The detection of the antibiotic was performed on a fluorescence detector (UltiMate 3000 Series), $\lambda_{eks.}$ – 254 nm $\lambda_{em.}$ – 560 nm. For analysis of the chromatograms the program «Chromeleon» (Dionex, USA) was used. The linearization curve was performed using the «Microsoft Excel» (Microsoft Corporation). Doxorubicin concentration in the serum was determined from the calibration curve in the concentration range from 50–500 ng/ml.

Statistical processing was performed on a personal computer with application programs «Statistica» (version 6.0). Experimental data are presented as mean

and error of the arithmetic m), which was calculated by analysis by variance using the±mean (M parametric criterion for assessing the reliability of (t-t-test).

Research was carried out in full compliance with the «Guidelines for conducting pre-clinical trials of drugs. Part One» (2012) [6] and Regulation Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation of 23.08.2010 № 708n). Animal experiments were performed in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123). Strasbourg, 1986). Studies carried out in accordance with approved written protocol plan preclinical studies and in accordance with standard operating procedures researcher (SOP).

Results and Discussion. Study of the most efficient use of time periods of ultrasound exposure, showed that in order to obtain the maximum amount of encapsulated doxorubicin niosomes, the required exposure time to ultrasound is 30 minutes (Table 1). Unilamellar niosomes with a size less than 100 nm with the inclusion of doxorubicin at over 58% were formed

Table 1

The content of doxorubicin niosomes at various time intervals ultrasonic's

Timeframe exposure ultrasound treatment (min.)	Power (W)	Number of doxorubicin (%)
15	150	51
30	200	63
45	250	61

Process steps for a pharmaceutical preparation based on gel doxorubicin niosomes organosilicon for the treatment of skin cancer are shown in Table 2.

Table 2

Phase preparation and pharmaceutical gel formulation based on silicone and doxorubicin niosomes for treating skin cancer

№ n/n	Phases of preparation and pharmaceutical gel formulation based on silicone and doxorubicin niosomes for treating skin cancer	
	Ingredient name	Contents
Phase A		
Intensive mechanical vibration on a shaker PEG-12 Dimethicone		
Phase B		
Ultrasonic treatment of the dispersion niosomes		
Phase C		
Mechanical stirring of the components in a mixer		
1	Purified water	up to 1000 ml
2	Doxorubicin	0,5 g
3	Gellant	50 ml
4	PEG-12 Dimethicone	100 ml
Phase D		
5	Triethanolamine	20 ml
Phase E		
	The emulsification of the gel on the AR homogenizer	

At the Figure presents a comparative analysis of the pharmacokinetics of doxorubicin when administered in the composition of niosomes (curve A) and in the free form (curve B), which showed that the introduction of antibiotic in its free form maximal concentration that was recorded in the first hours after administration. Beginning 6 hours after administration, the concentration level of doxorubicin in the blood decreases. It should be noted that the introduction of the free form of doxorubicin over the entire period of observation, its concentration is significantly lower compared with the administration of the doxorubicin niosomes composition. The maximum difference between the two forms of the drug was observed at the 12th hour of the study. At this point in time the experiment the concentration of doxorubicin when administered in a composition of niosomes is 5 times the control value. Despite a drastic reduction in the concentration of doxorubicin in the blood of the experimental animals at the end of our observation period at 48 hours after administration is a concentration of 3 times the control value.

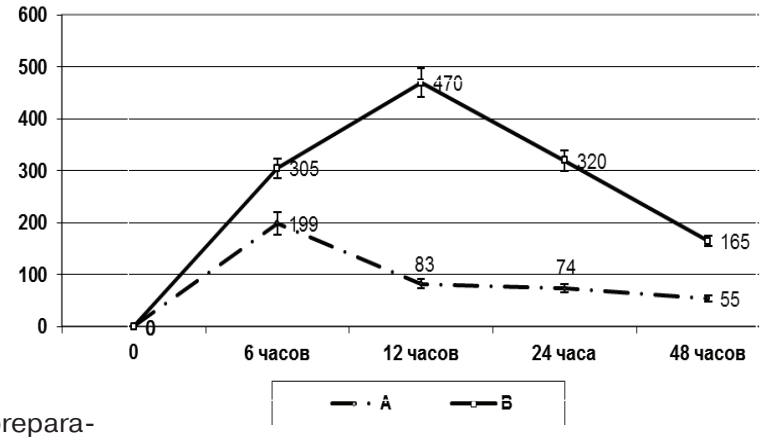

Fig. The average concentration values of doxorubicin (ng/ml) in the blood plasma of the experimental animals, when administered as part niosomes (B) or in the free form (A)

The experiment showed the high efficiency by the administrating doxorubicin as part of the niosomes. As evidenced by the data, niosomes form doxorubicin when using transdermal to facilitate a smooth increase in the concentration of the antibiotic in the blood along with the drug held in the blood for a long time at a consistently high level which is necessary for a sustained therapeutic effect.

As a result of this work, we have developed a pharmaceutical gel, which is are niosomes consisting of a shell of water-insoluble nonionic emulsifier double layer of PEG-12 Dimethicone, structurally similar to the basic structure of biological membranes, and retained inside the capsule of doxorubicin. Ultrasonic treatment of the dispersion niosomes led to an increase in the amount of doxorubicin in the interior of the niosomes.

Conclusions. A study of the pharmacokinetics of the gel with doxorubicin showed the prolonged effect of the drug caused by the gradual release of the drug, which allowed it to maintain high concentrations of the antibiotic in the blood for a long time. In addition, it is expected that a substantial increase in efficiency of

the drug developed by integrating the antibiotic organosilicon nanostructure – niosomes having substantial lipophilicity, thus by not sexposing doxorubicin in normal tissues, and only by penetration through the tumor capillaries of the skin defect. The chemical composition of niosomes provides deep transdermal penetration of drugs, in a size of less than 100 nanometers niosomes made invisible to the cells of the reticuloendothelial system, which increases the availability of the encapsulated drug to the target cells. Reduced heart and liver toxicity greatly expand the use of the drug among patients suffering from various chronic diseases. Application niosomal's gel will reduce the likelihood of necrosis at the injection site and other side effects of the drug.

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ROLE OF GENETIC FACTORS IN THERAPY WITH INDIRECT ANTICOAGULANTS IN ETHNIC GROUPS OF STAVROPOL REGION

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ЗНАЧЕНИЕ ГЕНЕТИЧЕСКИХ ФАКТОРОВ ДЛЯ ТЕРАПИИ НЕПРЯМЫМИ АНТИКОАГУЛЯНТАМИ В ЭТНИЧЕСКИХ ГРУППАХ СТАВРОПОЛЬСКОГО КРАЯ

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The study focused on specific issues about intake of indirect anticoagulants and the impact of the drug response genetic characteristics in representatives of the three ethnic groups residing in the Stavropol Region – Slavs, Armenians, and Karachais. The results showed that reaching the target value of INR in