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Original Article

THE DEVELOPMENT AND STUDY OF THE TOXICITY OF SUPPOSITORIES WITH A MODIFIED SUBSTANCE OF INTERFERON ALFA-2B

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ABSTRACT

Objective: To develop a stable composition with the substance of PEGylated interferon alfa-2b, to study technological and biopharmaceutical characteristics of the dosage form, and to conduct preclinical studies on the chronic toxicity and local irritating effect.

Methods: Solid fats Witepsol® H15, Witepsol® W 35, Suppocire® BS2X, and Suppocire® BM brands were used as the suppository bases. Polysorbate 80 was used as an emulsifier. Citric acid, ascorbic acid, sodium tetraborate, lactic acid, ethylenediaminetetetraacetic acid, tocopherol acetate was also introduced into the experimental samples. Fourteen experimental samples were screened for biological and technological indicators. Preclinical studies were performed for the optimal composition on the indicators of chronic toxicity and local irritant effect.

Results: The study examined the cytotoxic effects on the Vero cell line of selected suppository bases and excipients, namely, pH regulators and antioxidants. With excipients that did not have cytotoxicity we obtained suppository compositions with the following quality indicators: cytotoxicity, specific activity of interferon, time of complete deformation of suppositories and their melting temperature. A total of 14 compositions were studied, of which 5 were selected on the basis of the results for the study of stability. Only one composition turned out to be stable for the time studied.

Conclusion: The most stable in terms of "specific activity" was sample 7, its composition: Witepsol® H15/W35 70/30, polysorbate-80 0.15%, ethylenediaminetetraacetic acid (EDTA) 0.15%, sodium tetraborate 0.15%, tocopherol acetate 3.0%. Preclinical studies, that showed the absence of chronic toxicity and local irritant effect, were performed for this composition.

Keywords: Cytotoxicity, Interferon alfa-2b, Peginterferon, Specific activity, Suppositories

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INTRODUCTION

Indications for the use of interferon preparations come down to three main actions of this molecule: antiviral, immunostimulating and antiproliferative. In world practice there is a lot of experience in the use of, interferon alfa-2b for the treatment of infectious diseases in adults and children. Such diseases include respiratory infections and hepatitis. The use of, interferon alfa-2b allows in some cases to exclude the use of antibiotics and other antibacterial drugs, increases the effectiveness of antibacterial and antiviral therapy as part of complex therapy [1-4]. In addition, there is data on the effectiveness of interferon treatment of some types of cancer [5, 6]. Currently, studies are underway on the possibility of creating various modifications of interferon preparations that would make therapy more effective, highly specialized, and targeted [7, 8].

One of the directions for the modification of interferon is the introduction of external agents to improve the therapeutic properties [9, 10]. An example of the modification is the creation of PEGylated interferons by attaching molecules of polyethylene glycol (PEG) to them. PEGs of various molecular weights (from 300 to 5000 Daltons) are used; they have varying degrees of branching structure and are capable of covalently binding to protein molecules. The hydroxyl groups of PEG bind to the nitrogen atoms of the imidazole group of histidine and the amino groups of lysine and arginine, which makes it possible to order the structure of PEG and create an obstacle to the action of a complex molecule of enzymes: for example, trypsin, which reduces the rate of destruction of PEGylated interferon. In addition, PEG is highly hydrophilic and binds water molecules. This leads to the creation of a "water cloud" surrounding the complex of interferon and PEG, resulting in the increase of the solubility rate of the complex (which makes its bioavailability higher) and its protection from destructive external influences (neutralizing agents of the immune system, digestive enzymes). Due to this hydration barrier, the molecule breaks down more slowly and thus the effect of interferon becomes prolonged [11-13]. As confirmed by clinical studies, for example, PEGylated interferon alfa-2b was more effective in treating patients with chronic hepatitis C than interferon alfa-2b [14, 15].

The creation of suppositories seems promising as a delivery system for interferon. This dosage form has undoubted advantages, as it can be used in different age groups, including children, has a rapid release of the drug substance and high bioavailability [16-18].

The purpose of this study was to develop the composition and technology of suppositories with a new substance of PEGylated interferon alfa-2b, to study stability, technological and biopharmaceutical characteristics of the dosage form, and to conduct preclinical studies on the chronic toxicity and local irritating effect.

MATERIALS AND METHODS

The object of the study was the substance of modified PEGylated interferon alfa-2b (Binnopharm JSC, Russia), which has improved biopharmaceutical characteristics compared to interferon alfa-2b, in particular, greater stability, shelf life, better permeability, and bioavailability [2, 19].

Solid fats Witepsol® H15, Witepsol® W 35, Suppocire® BS2X and Suppocire® BM (EP 01/2009: 0462) were used as the basis for suppositories (table 1). Commercial rectal forms of interferon registered in the Russian Federation use cocoa butter substitute as a base. Which has a number of disadvantages, namely a narrow range of solidification temperatures and a high hydroxyl number.

Possibility of using the following substances as pH stabilizers and antioxidants was studied: citric acid (EP 01/2008: 0456 corrected 6.0), ascorbic acid (EP 01/2011: 0253), sodium tetraborate (EP 01/2008: 0013 corrected 6.0), lactic acid (EP 01/2008: 0458), ethylenediaminetetraacetic acid (EDTA) (USP-NF 1614239), tocopherol acetate (EP 07/2011: 0439), methionine (EP 01/2008: 1027 corrected 6.0).

Table 1: The compositions and characteristics of suppository bases

Name, manufacturer	Composition	Technological parameters, according to the manufacturer
Witepsol® H15 (IOI Oleo GmbH, Germany)	Solid fat (mixture of mono-, di-, triglycerides of vegetable acids C12-C18), the main part is triglycerides of lauric acid	Melting point of 33.5-35.5 °C; hydroxyl number 5-15 mg
Witepsol® W35 (IOI Oleo GmbH, Germany)	Solid fat (mixture of mono-, di-, triglycerides of vegetable acids C12-C18), the main part is triglycerides of lauric acid	Melting point of 33.5-35.5 °C; hydroxyl number 40-50 mg
Suppocire® BS2X Pellets (Gattefosse,France)	Solid fat consisting of mono-, di-and triglyceride esters of fatty acids (C10-C18), with a predominance of simple fraction and content of Polysorbate 65	The melting point of 35.0-39.0 °C; hydroxyl number 15-25 mg
Suppocire® BM Pellets (Gattefosse,France)	Solid fat consisting of mono-, di-and triglyceride esters of fatty acids (C10-C18), the dominant faction of triester	The melting point of 35.0-39.0 °C; hydroxyl number ≤ 10 mg

Suppositories were prepared by pouring into PVC molds: the pre-thermally sterilized base components were fused at a temperature of 45±1 °C, interferon alfa-2b was dissolved in a solution of pH stabilizers and antioxidants, also sterile (thermal sterilization), and introduced into the base with temperature 36±1 °C. Tocopherol acetate was administered directly by dissolving in the molten base. The mass of suppositories was 1.0 g, the dose of interferon alfa-2b per suppository was 1 000 000 IU.

The cytotoxicity of suppository ingredients was evaluated on a Vero cell line for two days. To form a cell monolayer a suspension of cells was poured into the wells of 96 well plates and incubated at 37±0.5 °C, 5% CO2 for 24 h. The test substances were mixed with the culture medium, incubated with constant stirring, filtered and then serial dilutions of samples in the culture medium were prepared. The lipophilic ingredients were dissolved in diethyl ether, then culture medium was added, mixed and diethyl ether was removed. With dispenser, samples were added to each well of tablets with Vero cells. Then, the tablet with cells was centrifuged and left to incubate at a temperature of 37±0.5 °C, 5% CO_2 for 2 d.

Assessment of the cytotoxic effect was carried out visually under a microscope (100-fold increase). The condition is considered non-toxic if the cell monolayer in the experimental well remains intact, without signs of degeneration, and does not differ from the control [20].

The specific antiviral activity of interferon in suppositories was determined by comparing the ability to suppress the cytopathic effect of the indicator virus in a Vero cell culture with a standard interferon sample (cat. number I0320301) according to EP requirements (Assay of interferons; 01/2008: 05600). The results were obtained with a visual method at a 100-fold increase. The antiviral activity of interferon in the test sample (X) in IU in 1 suppository was calculated by the formula:

$$X_{(IU\ /\ \text{sup})} = A_{ISS} \times \frac{Titer_{sample}}{Titer_{ISS}} \times C$$

Note:

A_{ISS}–activity of the international standard sample, 70000 IU;

C-the extraction coefficient of interferon from the suppository during extraction from the developed suppository bases was determined to be equal to 1.

The suppositories were sampled with diethyl ether: the suppositories were dissolved in diethyl ether, then the nutrient medium was added, the emulsion was mixed until homogeneous, centrifuged at 2000 rpm, and the solution was taken from the resulting lower hydrophilic layer for evaluation.

The suppository bases were analyzed for softening time determination according to the procedure described in EP (Softening time determination of lipophilic suppositories 01/2008: 20922) on an ERWEKA PM-30 instrument (ERWEKA, Germany). The melting point of a suppository was determined in accordance with the method of EP (01/2008:20215 Melting point–open capillary method). Microbiological purity was determined according to the pharmacopoeial requirements, *Escherichia Coli* should not be present in suppositories, the total number of aerobic microorganisms should not exceed 10^3CFU per 1 g and 10^2 CFU per 1 g for yeast and molds. Suppositories were stored at a temperature of+8-+10 °C.

The preclinical research of the developed composition of suppositories in terms of chronic toxicity and local irritant effects was conducted. The care and maintenance of animals were carried out in accordance with the recommendations and requirements of Directive 2010/63/EU of the European Parlament and of the EU Council of 22 September 2010 on the protection of animals used for scientific purposes. All animal experiments were approved by the Sechenov University Ethics Committee.

All *in vivo* studies were approved by the Ethics Committee of the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV", Moscow, Russia (approval No. 1095/20). Evaluation of the indicators was carried out on 20 males and 20 female rats weighing 180-220 g. Animals were purchased from Stolbovaya nursery for laboratory animals (Russia). All animals received care in accordance with the guidelines for accommodation and care of animals (ETS No. 123 "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes"). The animals were kept in a laboratory under veterinary observation in individual cages under normal conditions (room temperature of 22–24 °C; humidity of 30–70%), and a standard diet and water ad libitum were provided during the entire study. The dose of suppositories corresponding to the maximum therapeutic dose for humans (1,000,000 IU) was introduced daily for 30 d rectally. An autopsy was performed a day after the last injection. The Group of control animals consisted of 10 males and 10 females of the same mass. During the entire period of administration (within 30 d), the state and behavior of rats, visible physiological functions, the manifestation of symptoms of intoxication, animal death, etc. were carefully monitored. Every day the site of rectal administration of suppositories was carefully examined. Rat body weight was recorded daily. The functional state of the nervous system was assessed by visual observation of motor activity and behavioral reactions in the open field test. In this case, the rat was placed in the center of the illuminated area, divided into squares: the number of line crossings (locomotor activity), the look back number, the number of washings (grooming), the number of center square entries, the number of defecation acts (anxiety) were recorded. The functional state of the kidneys was assessed by diuresis for 3 h with an aqueous load of distilled water (2.5% relative to body weight) and clinical analysis of urine (by using an ANAGO SUB-NE refractometer (USA) and URS-20 test strips (USA).

The electrocardiographic examination was performed in rats at 0 (before the introduction of suppositories) and 1 d after the last injection. Electrocardiographic examination was performed on a 3-channel electrocardiograph EKZT-12-01 "Geolink" (Russia).

After performing intravital tests, the animals were euthanized by dislocation of the cervical vertebrae, blood samples were taken to determine hematological and biochemical parameters.

The main parameters of rat peripheral blood were determined on a MicroCC-20 Plus hematology analyzer (High Technology, Inc. (USA)) using the reagents of Clinical Diagnostic Solutions LLC (Russia); the leukocyte formula was determined by the conventional method. Blood biochemical parameters were determined on a Clima

MC-15 analyzer, RAL Technical el Laboratoria, S. A. (Spain) using the reagents manufactured by ZAO Deacon-DS (Russia).

Multiple organ macroscopic and microscopic examinations were carried out: liver, lungs, kidneys, heart, spleen, brain, lymph nodes, thymus, esophagus and stomach, small and large intestines, adrenal glands, pancreas, testes, ovaries, and rectal sites. The material was fixed in 10% formalin and embedded in paraffin. Histological slices were performed on a Microm HM325 microtome (Germany) and stained with hematoxylin-eosin. Microscopic preparations were examined under a microscope at a magnification of 90 × 10.

Results are expressed as mean±SEM. Data analysis was carried out using one-way ANOVA followed by Bonferroni post-tests using Microsoft Excel 2010 (Microsoft Corp. USA) and Statistica 8.0 for Windows (StatSoft Inc., USA) statistical analysis package. Significance was considered at values of $p<0.05$.

This research was carried out in compliance with all the relevant laws and institutional guidelines.

RESULTS AND DISCUSSION

Interferon alfa-2b has a wide spectrum of antiviral and immunomodulating effects, it is well absorbed when administered rectally and there are no toxic effects of interferon, in addition, it can be administered outside the hospital [21-23]. For the first time suppositories with a modified interferon substance were developed, they are stable during the storage and meet the requirements of national and European pharmacopoeias. Preclinical studies on the indicators of chronic toxicity and local irritant effect were conducted for the developed compositions. The prospects of further preclinical and clinical studies were shown.

The first stage of the study was the study of the cytotoxic effect of the selected bases for suppositories on the Vero cell line, the results are shown in table 2. Sample preparation of the bases was carried out with diethyl ether. Diethyl ether was chosen for sample preparation since it completely dissolves all the components of the suppository and then it can be removed without residue. The following method was used: 3.0 grams of a base or three suppositories at 37.0±1 °C on a shaker were dissolved in 10 ml of diethyl ether for 15 min, then an equal amount of culture medium was added and diethyl ether was removed in a vacuum oven, centrifuged at 2000 rpm and the lower liquid layer was analyzed. The bases should not have a cytotoxic effect in any of the dilutions.

Suppositories are supposed to include pH regulators and antioxidants, their cytotoxicity was also studied (table 3). All substances were applied to cells in the form of solutions in working concentration, except for tocopherol acetate, which was applied undiluted to the cells, but after sample preparation as for the bases for suppositories.

Table 2: The influence of bases for suppositories on the state of culture of cells

Dilution	Effect on cell culture samples					
	Witepsol® H15	Witepsol® W 35	Witepsol® H15/Witepsol® W 35 70/30	Witepsol® H15/Witepsol® W 35 50/50	Suppocire® BM	Suppocire® BS2X
1:10	-	-	-	-	-	++
1:20	-	-	-	-	-	+
1:40	-	-	-	-	-	+
1:80	-	-	-	-	-	+
1:160	-	-	-	-	-	-
1:320	-	-	-	-	-	-

Note: "-" no difference from control; "+" cytotoxicity missing, slight differences from the control; "++" cytotoxicity, the presence of dead cells, "+++" cytotoxicity, a large number of dead cells. *The table shows the mean value of 5 measurements.

Table 3: The influence of auxiliary substances on the state of culture of cells

Dilution	Effect on cell culture samples							
	Polysorbate 80 0.03%	EDTA 0.3%	Sodiumtetraborate 0.045%	Lactic acid 0.03%	Ascorbicacid 0.03 %	Citric acid 0.03%	Methionine 0.03%	Tocopherol acetate
1:10	+	+	-	++	+	+	+	+
1:20	-	-	-	+	-	+	+	+
1:40	-	-	-	-	-	-	-	+
1:80	-	-	-	-	-	-	-	+
1:160	-	-	-	-	-	-	-	-
1:320	-	-	-	-	-	-	-	-

Note: "-" no difference from control; "+" cytotoxicity missing, slight differences from the control; "++" cytotoxicity, the presence of dead cells, "+++" cytotoxicity, a large number of dead cells. *The table shows the mean value of 5 measurements.

The compositions of the suppository samples given in table 4 were developed based on the results of the data in tables 2 and 3. Compositions were prepared with all auxiliary substances in the concentrations corresponding to their cytotoxicity. Compositions 1-4 are obtained based on a mixture of Witepsol® H15/Witepsol® W35 in a ratio of 50/50, they differ in the content of pH stabilizers. Compositions 10-14 are similar to formulations 5-9, they differ in the basis, which here is Suppocire® BM. The content of Polysorbate 80 and EDTA is the same in all formulations and amounts to 0.0015 g per suppository.

Samples 1, 6, 8, 9, and 14 had a cytotoxic effect and were excluded from further studies. All the studied samples satisfy the pharmacopoeial requirements in the softening time determination and the melting point. For non-cytotoxic samples, the specific activity was determined, which should be in the range from 750 to 1250 thousand IU. Samples 5, 7, 10, 11, 12, and 13 satisfy this requirement. One of the main problems in the development of dosage forms of interferon is their stability, so it was studied in the next stage of work [24, 25]. Stability under storage conditions for 6 mo was studied in these samples. Samples were monitored by the following indicators: specific activity, softening time determination, melting point, and microbiological purity. The technological characteristics of the samples and microbiological purity did not change significantly during the studied storage period. Data on changes in the specific activity of experimental samples are given in table 6.

Table 4: The compositions of the developed suppositories

Composition № Components, g	Witepsol® H15/Witepsol® W35 50/50	Witepsol® H15/Witepsol® W35 70/30	Suppocire® BM	Polysorbate 80	EDTA	Lactic acid	Sodium tetraborate	Ascorbic acid	Citric acid	Tocopherol acetate	Methionine	Water for injections
1	to 1.0 g	-	-	0.0015	0.0015	0.0005	-	-	-	-	--	0.0005
2	to 1.0 g	-	-	0.0015	0.0015	-	0.0015	-	-	-	-	0 005
3	to 1.0 g	-	-	0.0015	0.0015	-	-	0.0005	-	-	-	0.0005
4	to 1.0 g	-	-	0.0015	0.0015				0.0015			0.0005
5	-	to 1.0 g	-	0.0015	0.0015	-	0.0015	-	-	-	-	0.0005
6	-	to 1.0 g		0.0015	0.0015	--	0.0015	0.0015	-	-	-	0.001
7	-	to 1.0 g		0.0015	0.0015	-	0.0015	-	-	0.03	-	0.001
8	-	to 1.0 g		0.0015	0.0015	-	0.0015	-	-	-	0.03	0.001
9	-	to 1.0 g		0.0015	0.0015	-	0.0015	-		0.03	0.03	0.001
10	-	-	to 1.0 g	0.0015	0.0015	-	0.0015	-	-	-	-	0.001
11	-	-	to 1.0 g	0.0015	0.0015	-	0.0015	0.0015	-	-	-	0.001
12	-	-	to 1.0 g	0.0015	0.0015	-	0.0015		0.03			0.001
13	-	-	to 1.0 g	0.0015	0.0015	-	0.0015				0.03	
14	-	-	to 1.0 g	0.0015	0.0015	-	0.0015	0.0015		0.03	0.03	

The next stage was the study of the characteristics of the obtained samples. The absence of the cytotoxic effect was studied firstly (table 5).

Table 5: Characteristics of the suppositories samples

№ composition	Cytotoxicity	The specific activity of interferon alfa-2b, thousand IU	Softening time determination, min	Melting point, °C
1	+	not determined	not determined	not determined
2	-	365.0±35.0	6.0±0.4	35.6±0.0
3	-	296.0±45.0	6.3±0.5	35.8
4	-	259.0±29.0	6.1±0.4	35.5
5	-	486.0±55.0	6.4±0.5	35.9
6	+	not determined	not determined	not determined
7	-	527.0±37.0	6.0±0.4	36.4
8	+	not determined	not determined	not determined
9	+	not determined	not determined	not determined
10	-	503.0±39.0	6.9±0.6	36.0
11	-	426.0±52.0	6.8±0.6	36.3
12	-	542.0±61.0	6.6±0.5	36.2
13	-	490.0±65.0	6.7±0.5	36.0
14	+	not determined	not determined	not determined

*The table shows the mean value of 5 measurements±SD

Table 6: The study of the stability of the suppositories samples

№ composition	The specific activity of interferon alfa-2b, thousand IU		
	Storage time		
	1 mo	3 mo	6 mo
5	1002.0±45.0	950.0±55.0	859.0±34.0
7	1020.0±35.0	996.0±50.0	978.0±54.0
10	952.0±38.0	862.0±48.0	751.0±51.0
11	898.0±37.0	852.0±46.0	743.0±35.0
12	857.0±45.0	759.0±37.0	698.0±48.0
13	901.0±51.0	667.0±50.0	713.0±52.0

*The table shows the mean value of 3 measurements±SD

The substance of PEGylated interferon alfa-2b requires special conditions for the development of the dosage forms based on it, due to the characteristics of the substance, the main method for analyzing the dosage form, and production technology. Interferon alfa-2b is relatively stable at a pH from 5.0 to 7.0, but slightly acidic values are preferred. The activity of interferon alfa-2b in the composition of dosage forms decreases during storage, therefore, antioxidants are introduced into the composition. The main method of analysis of the substance in the dosage forms is the determination of specific activity on cell culture; therefore, if the components of the dosage form have a cytotoxic effect the determination becomes impossible. There is also a number of requirements to the technology for the production of suppositories with interferon alfa-2b: the bases for suppositories must undergo sterilization and have a sufficiently large gap between the melting and crystallization temperatures so that the substance of interferon alfa-2b could be introduced to the suppository mass at a temperature not exceeding 37 °C. Filling of the suppositories should be also carried out at this temperature. The brands of solid fat of pharmacopoeial quality of the leading world manufacturers were selected as the base for suppositories. It is intended to use bases with a low hydroxyl number so that a significant amount of pH stabilizers is not required. Low hydroxyl number bases are contemplated to reduce the amount of added pH stabilizers. Witepsol solid fat brands are supposed to be used in combination, since Witepsol® H 15, despite the low value of the hydroxyl number, tends to be brittle,

suppositories obtained on its basis. To level this disadvantage, Witepsol® W 35 is introduced into the composition of the base. Support solid fat is distinguished by the presence of an emulsifier in Suppocire® BS2X, the use of which may not require additional administration of an emulsifier. As can be seen from table 2, Suppocire® BS2X solid fat has a significant cytotoxic effect at a 1:10 dilution and there are dead cells at 1:20 and 1:40 dilutions, therefore, no further studies on this brand were performed.

Based on the studies of the cytotoxicity of the components of the compositions, 14 suppository samples were developed, in which cytotoxicity, technological parameters, and specific activity after storage were determined.

In the sample of composition 7, the specific activity of interferon during six months of storage decreased by less than 10%, which is significantly lower compared to other samples. In samples 5 and 10-13, specific activity decreased by 14-21%. The selected composition and concentration of excipients increased the stability of the immunobiological substance in the dosage form, so this sample was chosen for the evaluation of the preclinical parameters. The composition was evaluated for chronic toxicity and local irritant effect.

During the entire period of rectal administration of the drug, animal death was absent; any signs of intoxication were not noted. The general condition of the animals remained without visible changes compared to the initial period and control animals. A daily examination of the injection site of the test drug in rats did not show swelling of the tissue, redness, increased local temperature, pain in the anus, or discharge from the rectum, which would indicate the presence of an inflammatory reaction. As the experiment progressed with an increase in the number of administrations, the state of the injection site did not change. The rectal administration of the drug did not have a significant effect on the mass gain of the rats and the mass ratios of rat organs.

Changes in hematological parameters caused by the introduction of the drug were reduced to an increase in the relative number of segmented neutrophils of 10.80±1.41% compared to the control (6.80±0.76%) and a decrease in the number of lymphocytes, 82.80±1.85% % compared to the control (88.50±1.36%), which is probably due to the pharmacological effect of the interferon and is the result of activation of hematopoiesis and is caused by compensatory reactions.

On the first day after the last administration of the drug, there was a statistically significant decrease in the activity of aspartate aminotransferase (236.50±9.86 compared to 273.40±9.98 U/L in the control). Alanine aminotransferase activity remained unchanged (59.30±14.40 in comparison with 62.30±3.60 U/L in the control). It is also necessary to note a decrease in the level of total bilirubin (4.16±0.33 compared to the control value of 8.20±1.32 μmol/L). In addition, the level of total protein decreased (68.00±1.95 compared to 74.50±1.10 g/L in the control) and the activity of lactate dehydrogenase decreased (2143.80±89.32 compared to 2552.00±84.73 U/L in the control). However, this is also associated with the pharmacological activity of the drug. A decrease in the concentration of total protein, bilirubin, and the activity of aspartate aminotransferase indicates the activation of protein metabolism. A decrease in the activity of lactate dehydrogenase is the result of increased oxidative processes and energy metabolism. The average values of the rhythm of heart contractions, the height of the teeth, and the duration of the intervals on the ECG in rats were comparable with those in the control animals and were in the range of physiological norms for rats.

Assessing the effect of the drug on the functional state of the central nervous system, it can be noted that during the period of drug administration rats retained motor activity and responses compared with the period before the experiment and observed in control animals. The administration of the drug in no case had a statistically significant effect on behavioral reactions, such as latency, crossings, rearings, grooming (frequency of grooming activity), and a number of defecations.

Assessing diuresis on the first day after the last injection of drugs, the indicators were: 6.92±0.35 against the control value of 6.27±0.61 ml. Renal function was also evaluated according to the results of the clinical analysis of rat urine after administration of the studied drug, the urine analysis data of experimental rats according to the results of quantitative and qualitative tests did not significantly differ from control values. Multiple organ macroscopic and microscopic examinations of the liver, lungs, kidneys, heart, spleen, brain, lymph nodes, thymus, esophagus and stomach, small and large intestines, adrenal glands, pancreas, testes, ovaries, rectal injection site demonstrated that after the last injection there were not any significant changes. All the revealed changes have functional and adaptive nature since there were no pathological changes during the macroscopic examination and on microscopic preparations of organs and tissues. Thus, the sample does not have chronic toxicity, although the experiment was carried out under harsh conditions (the maximum dose corresponding to the maximum therapeutic dose, the drug was administered 30 d, although the medication course is 5-7 d, possible repeated medication courses were taken into account). The studied sample of suppositories did not have a local irritant effect with repeated rectal administration. The results of preclinical studies of the suppositories correlate with the scientific data presented in publications. Thus, the study of acute toxicity in the oral administration of pegylated immobilized interferon showed very low toxicity [26]. Similar data were obtained in other studies on rectal dosage forms of interferon [27-28].

CONCLUSION

Based on the study of cytotoxicity on the Vero cell line, the ingredients were selected to develop the composition of suppositories with the PEGylated substance of interferon alpha-2b. Fourteen experimental samples were studied by the following indicators: cytotoxicity, the specific activity of the active substance, time of complete deformation, and melting point. Six samples were selected based on the results of the experiment, they were studied on the stability during the storage, the following indicators were monitored during the experiment: specific activity, time of complete deformation, melting point, and microbiological purity. Thus, the following composition of suppositories with PEGylated interferon was developed: interferon alfa-2b 1 million IU, polysorbate 80 0.015 g, EDTA 0.015 g, sodium tetraborate 0.015 g, tocopherol acetate 0.03 g, water for injection 0.001 g, Witepsol® H15/Witepsol® W35 70/30 to 1.0 g. The resulting composition meets the pharmacopoeial requirements for technological indicators and is stable for 6 mo of storage *in vivo*, does not possess chronic toxicity and local irritating effect. This composition is promising for the further study of stability to determine the shelf life of the dosage form and pharmacokinetic studies.

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All authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

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