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REPORT

Study of the Killevir preparation substance and medicinal form allergenic effect

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An allergenic effect of the Killevir preparation substance and two medicinal forms was studied: an injectional one (for the intravenous injections) and in a form of suppositoria (rectal). A substance of the Killevir preparation is a fullerenepolyaminocaproic acid.

An effect of the medicinal preparations on the experimental animals (mice of the Balb/c line and guinea-pigs-albinos) in a recommended for the clinical tests therapeutic dose (according to a substance content of 0,7 mg/kg for an injectional form and 0,14 mg/kg – for the suppositoria) and in a dose a cut above it was investigated. A scheme of sensibilization was selected according to the presumed in a clinic method of administration and according to the "Methodical instructions on the pharmacologic substance allergenic property assessment" of the Russian federation (RF) Ministry of Public Health (MPH) Pharmaceutic Committee (PhC). A state of a hypersensitivity of the immediate and delayed types to the medicinal preparations was revealed: in a test of a paw edema (HDT, of a mouse), a response of an active cutaneous anaphylaxis (HIT, of a mouse), by a method of a conjunctival test (guinea-pigs) and in a response of a general anaphylaxis (HIT, of the guinea-pigs). The intact animals and animals receiving placebo according to a scheme of the medicinal preparation administration served as the control groups.

It was established, that the investigating medicinal preparations in a dose, ten-fold exceeding the therapeutic one, were leading to a HIT forming, which was manifested in a death of the guinea-pigs 20% from an anaphylactic shock, at the following schemes of sensibilization: a substance and the Killevir preparation (injectional form) – a three-fold administration (in a day: subcutaneously and twicely - intramuscularly), the Killevir preparation (the rectal suppositoria) – a multiple rectal administration (once a day, by 5 days in a week, totally 4 weeks).

A substance and the both medicinal forms of the Killevir preparation did not form a hypersensitivity of the immediate or delayed types in mice and guinea-pigs in the recommended therapeutic doses for the clinical tests.

Thus, an individual sensitivity to an allergenic effect of the Killevir preparation substance and injectional form high doses (7,0 mg/kg) as well as the suppository rectal Killevir preparation (at a durative administration in a dose of 1,4 mg/kg) is possible.

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INTRODUCTION

A hypersensitivity state forming to any substance, being a part of the preparation, relates to the possible side effects, appearing at a use of the medicinal preparations. A detection of such effects in the elaborating pharmacologic preparations is an obligatory stage of their preclinical assessment.

An aim of the present investigation is a detection of the Killevir preparation substance and both medicinal form allergenic properties. The preparation is presumed to be used as an antiviral, specifically, antiherpetic medicinal preparation.

A substance of the Killevir preparation is a fullerenepolyaminocaproic acid.

A composition of the Killevir preparation (an injectional form, concentrate; to dissolve in 30 ml of water for injections before infusion):

- a fullerenepolyaminocaproic acid – 50 mg;
- a dimethylsulfoxide (DMSO, Dimexide PhS) – 1 ml;
- a water for injections – 2 ml

A composition of the Killevir preparation (suppositoria rectal):

- a fullerenepolyaminocaproic acid – 10 mg;
- a dimethylsulfoxide (DMSO, Dimexide PhS) – 200 mg;
- a vitepsol – up to 2 g.

The work is accomplished according to the “Methodical instructions on assessment of the pharmacologic substance allergenic properties” of the RF MPH Pharmacologic committee [2].

1. MATERIALS AND METHODS

The work is made according to the methodical instructions [2].

Laboratory animals

The experiments are made on mice of the Balb/c line (a body mass is 26±2 g) and on the guinea-pigs-albinos (a body mass is 380±40 g). The animals are acquired in the nurseries "Stolbovaya" (mice) and "Kryukovo" (guinea-pigs). A maintenance of the animals in a vivarium of the TBP SRC corresponded to the sanitary rules, approved by the USSR MPH on 73.07.06, on arrangement, equipping and maintenance of the experimentally-biological clinics (vivaria) [1].

The rodents were fed with a full-rational mixed feed and they drank a plumbing water. The animals have passed a quarantine and acclimatization in the vivarium conditions during not less than 14 days.

The experimental groups of animals were formed by a method of a random selection considering a body mass as a leading value.

Preparation of the investigating pharmacologic preparation solutions

Presented for investigation:

1) a substance of the Killevir preparation (a fullerenepolyaminocaproic acid);
2) a concentrate of the Killevir medicinal preparation (injectional form) of the following composition:
    - a fullerenepolyaminocaproic acid – 50 mg,
    - a dimethylsulfoxide (DMSO, Dimexide PhS) – 1 ml,
    - a water for injections – 2 ml; to dissolve a concentrate in 30 ml of a water for injections before infusion;
3) the Killevir medicinal preparation (suppositoria rectal) of the following composition:
    - a fullerenepolyaminocaproic acid – 10 mg,
    - a dimethylsulfoxide (DMSO, Dimexide PhS) – 200 mg,
    - a vitepsol – up to 2 g.

The medicinal form solutions for infusion were prepared according to the rules of antiseptics and aseptics in a laminar box at a vertical flow of a sterile air.

Substance

A concentrate of a substance solution was thus prepared in a DMSO, that a rated dose was administered to a DMSO 3% at a further dilution in a physiologic salt solution.

Medicinal preparation Killevir (injectional form)

A therapeutic dose of the Killevir preparation, presumed for the tests in a clinic (the injectional forms are according to the substance content), is 0,7 mg/kg (1 TD); a dose, ten-times exceeding it, is 7 mg/kg (10 TD).

A concentrate (3 ml) was dissolved in 30 ml of a water for injections before infusion, the received solution is a solution A.

10 TD:

- At a mouse body mass of 20 g it is 0,14 mg/specimen (according to the substance content). 0,14 mg of a substance is contained in 0,08 ml of the Solution A. By 0,08 ml of the Solution A was infused into each specimen at a body mass of 20 g.
- At a guinea-pig body mass of 330 g it is 2,3 mg/specimen (according to the substance content). 2,3 mg of a substance is contained in 1,4 ml of the Solution A. 1,4 ml of the Solution A, at a body mass of 330 g, was infused into each specimen into three points: 0,5 ml, 0,5 ml and 0,4 ml.

1 TD:

- At a mouse body mass of 20 g, it is 0,014 mg/specimen (according to a substance content). After the Solution A 10-fold dilution with a use of a DMSO 3% (the received solution B), 0,014 mg of a substance is contained in 0,08 ml of the Solution B. By 0,08 ml of the Solution B was infused into each specimen at a body mass of 20 g.
- At a guinea-pig body mass of 330 g, it is 0,23 mg/specimen (according to a substance content). 0,23 mg of a substance is contained in 0,14 ml of the Solution A. By 0,14 ml of the Solution A was infused into each specimen at a body mass of 330 g.

Medicinal preparation Killevir (suppositoria rectal)

The presumed for the tests in a clinic therapeutic dose of the Killevir preparation (suppositoria rectal, according to a substance content) is 0,14 mg/kg (1 TD); a dose, 10 times exceeding it (10 TD), is 1,4 mg/kg.

10 TD:

- At a mouse body mass of 20 g, it is 0,028 mg/specimen (according to a substance content), the pointed dose is contained in 5,6 mkl of a medicinal form. By 6,0 mkl of the Killevir preparation were infused into each specimen at a body mass of 20 g.
- At a guinea-pig body mass of 330 g, it is 0,467 mg/specimen (according to a substance content), the pointed dose is contained in 93 mkl of a medicinal form. By 95 mkl of the Killevir preparation were infused into each specimen at a body mass of 330 g.

1 TD:

- At a mouse body mass of 20 g, it is 0,0028 mg/specimen (according to a substance content), the pointed dose is contained in 0,56 mkl of a medicinal form. The medicinal form was 10 times diluted with a use of placebo for a rectal form (Dilution C), infused by 6,0 mkl of the Dilution C into each specimen at a body mass of 20 g.
- At a guinea-pig body mass of 330 g, it is 0,047 mg/specimen (according to a substance content), the pointed dose is contained in 9,3 mkl of a medicinal form. The medicinal form was 10 times diluted with a use of placebo for a rectal form (Dilution C), infused by 95 mkl of the Dilution C into each specimen at a body mass of 330 g.

Receipt of a substance or the Killevir preparation suspension (injectional form) in the Freind's full adjuvant (FFA)

1) Receipt of the FFA working dilution (dFFA): the FFA was mixed with a physiologic salt solution in a ratio of 1:1;

2) two parts of the investigating pharmacologic preparation corresponding dilution – 0,04 ml and 0,08 ml, respectively, (at a mouse body mass of 20 g) were added to a part of the received dFFA (thoroughly mixing);

3) the received emulsions of a pharmacologic preparation (by 0,12 ml at a mouse body mass of 20 g) were subcutaneously infused (into the inguinal area).

Placebo

1) A placebo for an injectional form is a DMSO 3% solution, prepared on a water for injections. By 0,08 ml to a specimen at a body mass of 20 g (of a mouse) or by 1,4 ml at a body mass of 330 g (guinea-pigs) were infused

2) A placebo for suppositoria:

- a dimethylsulfoxide (DMSO, Dimexide PhS) – 200 mg,
- a vitepsol – up to 2 g.

It was infused by 6,0 mkl to a specimen at a body mass of 20 g (of a mouse) or by 95 mkl to a specimen at a body mass of 330 g (guinea-pigs).

Detection of a hypersensitivity of a delayed type in a test of a mouse paw edema

A HDT was revealed in 5 days after the last infusion of a substance or a medicinal preparation. The resolving dose, dissolved in a DMSO 3%, was infused into the posterior paw pillow in a volume of 0,05 ml, 0,05 ml of a DMSO 3% - into the opposite paw – the control one.

In 24 hours the HDT index of reaction (IR) was detected according to a difference in weight of the experimental (D_{ex}) and control (D_c) paws according to the formula:

$$IR = \frac{D_{ex} - D_c}{D_c} \times 100$$

A resolving dose of a sensibilizing agent – a dose, causing no intense non-specific inflammation at infusion into the intact mice (IR is up to 10%), was preliminary selected.

The meanings of the IR values in the experimental and control groups of the animals were compared.

Detection of a hypersensitivity of the immediate type (HIT) in mice in a reaction of an active cutaneous anaphylaxis (ACA)

A HIT was revealed in 14 days after the last sensibilization. The resolving doses of a substance or a medicinal form were subcutaneously infused into the animals in a DMSO 3% in a volume of 0,05 ml at a conduction of the ACA reaction; 0,05 ml of a DMSO 3% was infused into the control points. In 20 min.,

0,2 ml of the Evans' blue stain solution 1% was injected into a caudal vein of the mice. In 30 min, the animals were killed and the reaction was assessed according to a diameter of the exudate spot, stained with the Evans' blue on an internal surface of the skin in areas of the resolving dose infusion.

The maximum resolving doses of a substance or a medicinal form, causing no non-specific increase of the blood capillary permeability, were preliminary selected on the intact mice prior to a HIT detection.

A difference in dimensions of the exudate spots, formed in response to the resolving dose infusion in animals from the experimental and control groups was assessed.

Method of a conjunctival test

A resolving dose of a substance or a medicinal form in a DMSO 3% was infused with an ocular pipette with a stretched thin end under the upper lid into the experimental and control guinea-pigs. A drop of a DMSO 3% was infused into the second eye (the control one).

A reaction has been considered during the first 15-30 min. (a rapid reaction) and in 6-24-48 hours (a hypersensitivity of a delayed type) and assessed according to the following scale (in marks):

1. – a light reddening of a tear duct;
2. – a reddening of a tear duct and a sclera in direction of a cornea;
3. – a reddening of all the conjunctiva and sclera.

A selection of the substance or medicinal form resolving doses was made by dropping of the different concentrations into an eye of the unsensibilized guinea-pigs.

Reaction of a general anaphylaxis (anaphylaxis shock)

The Killevir preparation (injectional form) was intracardiacly infused into the experimental and control guinea-pigs in a volume of 0,75 ml, which was 1,25 mg according to a substance content. A state of the animals has been registered during an hour.

A consideration of the anaphylactic shock intensity was made in the indices according to Weigle.

Statistical treatment of the data

A statistical treatment of the received experimental data was conducted with a use of the Student's criterium [3].

2. RESULTS OF INVESTIGATIONS

An allergenic activity of the pharmacologic preparations is manifested in a hypersensitivity state forming to them. A hypersensitivity of a delayed type (HDT), stipulated by a presence of the sensibilized lymphocytes, and a hypersensitivity of the immediate type (HIT), stipulated by a presence of the humoral factors, are differentiated. An allergenic effect of a substance and the two medicinal forms of the Killevir preparation was studied on two types of the laboratory animals: the mice and guinea-pigs.

A selection of the investigated doses is stipulated by recommendations of the elaborator: the presumed at a use in a clinic therapeutic dose (1 TD) of the Killevir preparation (injectional form) is 0,7 mg/kg (according to a substance content), of the Killevir preparation (suppositoria rectal) is 0,14 mg/kg (according to a substance content).

2.1 STUDY OF THE KILLEVIR SUBSTANCE AND MEDICINAL PREPARATION (INJECTIONAL FORM) ALLERGENIC EFFECT

An effect of the two levels of doses was studied: the presumed to a use in clinic therapeutic dose and a dose, 10 times exceeding it.

2.1.1. STUDY OF A HDT FORMING ON MICE

An ability of the Killevir substance and medicinal preparation (injectional form) to form a HDT was studied on the Balb/c line mice at a sensibilization according to the methodical recommendations [2], with a following detection of a HDT in a test of a paw edema.

The mice were once subcutaneously sensibilized in the Freind's full adjuvant (FFA) in the doses of 0,7 mg/kg and 7,0 mg/kg (according to a substance content). The control groups №1 are presented by the animals, sensibilized with placebo in the FFA (DMSO 3%), the control groups №2 are presented by the intact animals, receiving only the FFA. By 10 animals at an equal ratio according to a sex were taken to each experimental and control groups.

The preliminary selected resolving doses of the investigating medicinal preparations, causing no non-specific inflammation in the intact mice, were 100 mkg (according to a substance content, dissolved in a DMSO 3%). A HDT was revealed in 24 hours after the resolving dose infusion.

The results of the conducted investigation are presented in a table 1. The values of the HDT IR in the groups of mice, receiving the testing medicinal preparations, and in the control groups were not different. A substance and the Killevir preparation (injectional form) in a therapeutic dose and in a dose, 10-times exceeding it, did not cause a hypersensitivity of a delayed type forming in mice.

Table 1.

Values of a hypersensitivity of a delayed type reaction in mice after a single subcutaneous sensibilization

Testing medicinal preparation	Dose, mg/kg	HDT IR, %, M±m
Substance	0,7	3,88±0,60
	7,0	4,13±0,75
	Control №1 (placebo)	3,92±0,61
	Control №2 (intact)	3,72±0,76
Killevir (injectional form)	0,7	3,51±0,91
	7,0	3,54±1,02
	Control №1 (placebo)	3,65±0,94
	Control №2 (intact)	3,42±0,61

2.1.2. STUDY OF A HIT FORMING IN MICE

An ability of the Killevir substance and medicinal preparation (injectional form) to form a HIT was studied on mice of the Balb/c line at a sensibilization according to the methodical recommendations [2], with a following detection of a HIT in a reaction of an active cutaneous anaphylaxis (ACA).

The mice were sensibilized with each of the testing medicinal preparations according to the following scheme: the first sensibilization was subcutaneously, the second and third were intramuscularly in a day. The daily doses were 0,7 mg/kg and 7,0 mg/kg (according to a substance content). The control groups №1 were presented by the animals, sensibilized with a placebo, the control groups №2 were presented by the intact animals. By 10 animals at an equal ratio according to a sex were taken to each experimental and control groups.

The preliminary selected maximum resolving doses of the investigating medicinal preparations, causing no non-specific increase of the blood capillary permeability in the intact mice, were 100 mkg (according to a substance content) in 0,05 ml of a DMSO 3%. A HIT was detected in 14 days after the last sensibilization. 100 mkg (according to a substance content) of a medicinal preparation were infused intracutaneously into two points at a conduction of the ACA reaction, 50 mkg (according to a substance content) of a medicinal preparation were infused into the third point and 0,05 ml of a DMSO 3% was infused into the fourth one.

It appeared, that the dimensions of the exudate spots in the sensibilized mice, formed in the points of the resolving dose infusion, did not reach 2 mm in a diameter and did not exceed the exudate spot dimensions in the control points. Thus, a hypersensitivity of the immediate type in the ACA reaction in mice is not revealed.

A substance and the Killevir preparation (injectional form) in a therapeutic dose and in a dose, 10-times exceeding it, did not cause a hypersensitivity of the immediate type forming in mice.

2.1.3. DETECTION OF A SENSIBILIZATION BY A CONJUNCTIVAL TEST METHOD IN THE GUINEA-PIGS

An allergenic effect of the Killevir substance and medicinal preparation (injectional form) was studied on the guinea-pigs-albinos at the animal testing by a conjunctival test method.

The guinea-pigs were sensibilized with each of the testing medicinal preparations according to the following scheme: the first sensibilization was subcutaneously, the second and third were intramuscularly in a day. The daily doses were 0,7 mg/kg and 7,0 mg/kg (according to a substance content). The control groups №1 were presented by the animals, sensibilized with a placebo, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups.

As a result of a preliminary selection of the investigating medicinal preparation dilutions, causing no non-specific inflammation in the intact guinea-

pigs, it was decided to use the Killevir preparation (injectional form) and a substance solution in a DMSO 3% as the resolving doses in the same concentration as in the preparation.

A conjunctival test was made in 14 days after the last sensibilization. As a result, none of the experimental animals with a rapid (during the first 15-30 min.) or delayed (in 6-24-48 hours) reaction to the investigating medicinal preparations has been revealed. A state of the tear duct, sclera and conjunctiva upon the whole was without alterations after the resolving dose infusion.

Thus, an allergenic effect of the Killevir substance and preparation (injectional form) taken in a therapeutic dose and in a dose, 10-times exceeding it, is not established by a conjunctival test method on the guinea-pigs.

2.1.4. DETECTION OF A HIT IN A GENERAL ANAPHYLAXIS REACTION IN THE GUINEA-PIGS

An allergenic effect of the Killevir substance and medicinal preparation (injectional form) was studied on the guinea-pigs-albinos at the animal testing in a general anaphylaxis reaction.

The guinea-pigs were sensibilized with each of the testing medicinal preparations according to the following scheme: the first sensibilization was subcutaneously, the second and third were intramuscularly in a day. The daily doses were 0,7 mg/kg and 7,0 mg/kg (according to a substance content). The control groups №1 were presented by the animals, sensibilized with a placebo, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups.

A HIT was revealed in 21 days after the last sensibilization. A resolving dose of the Killevir preparation (injectional form) was infused into the experimental and control guinea-pigs intracardiacly in a volume of 0,75 ml, which was 1,25 mg, according to a substance content, an observation of the animals has been conducted during an hour.

A resolving dose must exceed a total sensibilizing dose. This condition is accomplished for the guinea-pig groups, sensibilized with a therapeutic dose (1 TD) of the medicinal preparation, at an infusion of the preparation pointed volume.

There was no possibility to increase a resolving dose for the guinea-pig groups, sensibilized with a 10 TD dose: the intact guinea-pigs did not sustain an increase of the infusing preparation volume. A DMSO content was increasing to 6% at a substance concentration increase by a 5-times dilution of a concentrate for injections and not a 10-times one, according to instruction, besides, the intact animals died at an intracardiac infusion of a substance 0,3-0,5 ml into a DMSO 6%.

As a result of the conducted investigation, none of the animals with the anaphylactic shock manifestations in the guinea-pig groups, sensibilized with the medicinal preparations in a therapeutic dose, was revealed. By 2 of the 10 guinea-pigs have died during 10 min. after an intracardiac infusion of a resolving dose in the groups of animals, sensibilized with the medicinal preparations in a ten-fold therapeutic dose (Table 2). The observed manifestations: a lateral position (in 5-9

min), a hurried breathing and death during 30 sec. after the first signs of the anaphylactic shock. At a following autopsy of the died animals, a puncture site in the heart was observed, a thoracic cavity was without blood. The signs, accompanying the anaphylactic shock, were absent in the other animals from these experimental groups (by 8 of 10). A response reaction to a resolving dose infusion is not noted in the control groups.

Table 2.

Results of a general anaphylaxis reaction conduction on the guinea-pigs, sensibilized with a substance or the Killevir preparation (injectional form)

Testing medicinal preparation	Dose, mg/kg	Received data	
		Number of animals in a group	Died
Substance	0,7	10	0
	7,0	10	2
	Control №1 (placebo)	10	0
	Control №2 (intact)	5	0
Killevir (injectional form)	0,7	10	0
	7,0	10	2
	Control №1 (placebo)	10	0
	Control №2 (intact)	5	0

Thus, an allergenic effect of the Killevir substance and preparation (injectional form), taken in a dose, ten-times exceeding a therapeutic one, manifested in 20% of animals in a death from the anaphylactic shock, is established on the guinea-pigs. The Killevir substance and preparation (injectional form) in a therapeutic dose (0,7 mg/kg) did not lead to a hypersensitivity of the immediate type development, a reaction of a general anaphylaxis at an intracardiac infusion of the resolving doses was not developing in the guinea-pigs.

2.2. STUDY OF THE KILLEVIR MEDICINAL PREPARATION ALLERGENIC EFFECT (SUPPOSITORIA RECTAL)

An effect of the two levels of doses: the presumed for a use in a clinic therapeutic dose and a dose, 10-times exceeding it, was studied.

2.2.1. STUDY OF THE HDT FORMING ON MICE

An ability of the Killevir medicinal preparation (suppositoria rectal) to form a HDT was studied on mice of the Balb/c line at a multiple rectal infusion, with a following detection of a HDT in a test of a paw edema. The preparation was infused in doses of 0,14 mg/kg and 1,40 mg/kg (according to a substance content) according to the following scheme: once a day, by 5 days a week, totally 4 weeks.

The control groups №1 were presented by the animals, sensibilized with a placebo for a rectal form according to the same scheme, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups at an equal ratio according to a sex.

A preliminary selected resolving dose, causing no non-specific inflammation at an infusion into the intact mice, was 100 mkg of a substance (dissolved in a DMSO 3%).

A HDT was assessed in 24 hours after a resolving dose infusion. The results of the conducted investigation are presented in table 3. The meanings of the HDT IR in the groups of mice, receiving the Killevir preparation (suppositoria rectal), and in the control groups were not different. The Killevir preparation (suppositoria rectal) in a therapeutic dose and in a dose, 10-times exceeding it, did not cause a hypersensitivity of a delayed type forming in mice.

Table 3.

Values of a hypersensitivity of a delayed type reaction in mice after a multiple – during 4 weeks – rectal infusion of the Killevir preparation

Testing medicinal preparation	Dose, mg/kg	HDT IR, %, M±m
Killevir (suppositoria rectal)	0,14	4,77±1,45
	1,40	5,21±1,10
	Control №1 (placebo)	5,52±1,16
	Control №2 (intact)	5,32±1,05

2.2.2. STUDY OF A HIT FORMING ON MICE

An ability of the Killevir medicinal preparation (suppositoria rectal) to form a HIT was studied on mice of the Balb/c line at a multiple rectal infusion, with a following detection of a HIT in a reaction of an active cutaneous anaphylaxis. The preparation was infused in doses of 0,14 mg/kg and 1,40 mg/kg (according to a substance content) according to the following scheme: once a day, by 5 days a week, totally 4 weeks. The control groups №1 were presented by the animals, sensibilized with a placebo for a rectal form according to the same scheme, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups at an equal ratio according to a sex.

The preliminary selected maximum resolving dose, causing no non-specific increase of the blood capillary permeability in the intact mice, was 100 mkg of a substance (in a DMSO 3%). A HIT was revealed in 14 days after the last sensibilization. The resolving doses were infused into three points at the ACA reaction conduction: 100 mkg, 100 mkg and 50 mkg (in a volume of 0,05 ml): 0,05 ml of a DMSO 3% was infused into the fourth-control point.

It appeared, that the dimensions of the exudate spots in the sensibilized mice, formed in the points of the resolving dose infusion, did not reach 2 mm in a diameter and did not exceed the dimensions of the exudate spots in the control points. Thus, a hypersensitivity of the immediate type is not revealed in the ACA reaction in mice.

The Killevir preparation (suppositoria rectal) in a therapeutic dose and in a dose, 10-times exceeding it, did not cause a hypersensitivity of the immediate type forming in mice at a multiple (during 4 weeks) rectal infusion.

2.2.3. DETECTION OF A SENSIBILIZATION BY A CONJUNCTIVAL TEST METHOD IN THE GUINEA-PIGS

An allergenic effect of the Killevir medicinal preparation (suppositoria rectal) was studied on the guinea-pigs-albinos at the animal testing by a conjunctival test method.

The guinea-pigs were sensibilized by a multiple rectal infusion of the preparation in doses of 0,14 mg/kg and 1,40 mg/kg (according to a substance content). A scheme of infusion: once a day, by 5 days a week, totally 4 weeks. The control groups №1 were presented by the animals, sensibilized with a placebo for a rectal form according to the same scheme, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups.

As a result of the resolving dose preliminary selection, causing no non-specific inflammation at an infusion into the intact guinea-pigs, a substance solution in a DMSO 3% in a concentration of 1,67 mg/ml is selected.

A conjunctival test was made in 14 days after the last sensibilization. As a result, none of the experimental animals with a rapid (during the first 15-30 min.) or delayed (in 6-24-48 hours) reaction to a resolving dose was revealed: a state of the tear duct, sclera and conjunctiva upon the whole was without alterations.

Thus, an allergenic effect of the Killevir preparation (suppositoria rectal) is not established by a conjunctival test method on the guinea-pigs at a multiple use (during 4 weeks) in a therapeutic dose and in a dose, 10-times exceeding it.

2.2.4. DETECTION OF A HIT IN A GENERAL ANAPHYLAXIS REACTION IN THE GUINEA-PIGS

An allergenic effect of the Killevir medicinal preparation (suppositoria rectal) was studied on the guinea-pigs-albinos at the animal testing in a general anaphylaxis reaction.

The guinea-pigs were sensibilized by a multiple rectal infusion of the preparation in doses of 0,14 mg/kg and 1,40 mg/kg (according to a substance content). A scheme of infusion: once a day, by 5 days a week, totally 4 weeks. The control groups №1 were presented by the animals, sensibilized with a placebo for a rectal form according to the same scheme, the control groups №2 were presented by the intact animals. By 10 animals were taken to each experimental and control groups.

A HIT was revealed in 21 days after the last sensibilization. 1,25 mg of a substance (in a DMSO 3%) in a volume of 0,75 ml was infused into the experimental and control guinea-pigs intracardiacly, an observation of the animals has been conducted during an hour. A resolving dose must exceed a total sensibilizing dose. This condition is accomplished for the guinea-pig groups, sensibilized with the preparation in a therapeutic dose, there was no possibility to

increase a resolving dose for the ones, sensibilized with the preparation in a dose of 10 TD.

As a result of the conducted investigation, none of the animals with the anaphylactic shock manifestations in the guinea-pig groups, sensibilized with the preparation in a therapeutic dose, was revealed. Two of ten guinea-pigs have died during 10 min. after a resolving dose intracardiac infusion in a group of animals, sensibilized with the preparation in a ten-fold therapeutic dose (Table 2). The observed manifestations: a lateral position (in 5-9 min), a hurried breathing and death during 30 sec. after the first signs of the anaphylactic shock, a puncture site in the heart is observed at an autopsy, a thoracic cavity is without blood. A response reaction to a resolving dose was absent in the other 8 guinea-pigs from that experimental group, a response reaction in the control groups to a resolving dose infusion is not noted.

Table 4.

Results of a general anaphylaxis reaction conduction on the guinea-pigs, sensibilized with the Killevir preparation (suppositoria rectal)

Testing medicinal preparation	Dose, mg/kg	Received data	
		Number of animals in a group	Died
Killevir (suppositoria rectal)	0,14	10	0
	1,40	10	2
	Control №1 (placebo)	10	0
	Control №2 (intact)	10	0

Thus, an allergenic effect of the Killevir preparation (suppositoria) is established on the guinea-pigs at a multiple (during 4 weeks) rectal infusion in a dose, 10-times exceeding a therapeutic one, manifested in a death from the anaphylactic shock of the animals 20%. The Killevir preparation (suppositoria rectal) in a therapeutic dose (0,14 mg/kg) at a multiple (during 4 weeks) infusion did not lead to a hypersensitivity of the immediate type development: a general anaphylaxis reaction at an intracardiac infusion of a resolving dose was not developing in the guinea-pigs.

CONCLUSION

A study of an allergenic effect of a substance and the two medicinal forms of the Killevir preparation: injectional one (for the intravenous injections) and in a form of suppositoria (rectal) is made.

The Killevir preparation substance is a fullerenepolyaminocaproic acid.

A composition of the Killevir preparation (injectional form, concentrate; to dissolve in 30 ml of water for injections before infusion):

- a fullerenepolyaminocaproic acid – 50 mg,
- a dimethylsulfoxide (DMSO, Dimexide PhS) – 1 ml;

- a water for injections – 2 ml.

A composition of the Killevir preparation (suppositoria rectal):

- a fullerenepolyaminocaproic acid – 10 mg,
- a dimethylsulfoxide (DMSO, Dimexide PhS) – 200 mg,
- a vitepsol – up to 2 g.

A selection of the investigated doses is stipulated by recommendations of the elaborator: the presumed therapeutic dose of the Killevir preparation (injectional form) is 0,7 mg/kg (according to a substance content), of the Killevir preparation (suppositoria rectal) is 0,14 mg/kg (according to a substance content).

The experimental animals – the mice of the Balb/c line and the guinea-pigs-albinos were sensibilized according to the presumed in a clinic way of the medicinal preparation infusion. An effect of the two levels of doses was tested: the recommended one for the clinical tests (a therapeutic dose) and the one, 10-times exceeding it. A state of a hypersensitivity of the immediate and delayed types to the medicinal preparations was revealed: in a test of a paw edema (HDT, of a mouse), in a reaction of an active cutaneous anaphylaxis (HIT, of a mouse), by a conjunctival test method (guinea-pigs) and in a general anaphylaxis reaction (HIT, guinea-pigs). The control groups were presented by the intact animals and the animals, receiving a placebo according to a scheme of the medicinal preparation infusion.

It was established, that a substance and the two medicinal forms of the Killevir preparation in doses, 10-times exceeding a therapeutic one, were leading to a hypersensitivity of the immediate type forming, manifested in a death of the guinea-pigs 20% from the anaphylactic shock at the following schemes of sensibilization:

- the Killevir substance and preparation (injectional form) – a three-fold infusion (in a day: subcutaneously, and twicely - intramuscularly) in a dose of 7,0 mg/kg;
- the Killevir preparation (suppositoria rectal) – a multiple rectal infusion (once a day, by 5 days a week, totally 4 weeks) in a dose of 1,4 mg/kg.

A hypersensitivity of a delayed type at an infusion of the ten-fold therapeutic doses of the investigating medicinal preparations in the experimental animals is not revealed.

According to the “Methodical instructions on assessment of the pharmacologic substance allergenic properties” [2], the observed effects are regarded as a manifestation of an individual sensitivity, if a number of the sensibilized animals in a group according to one of the tests is less than 50%. Thus, an allergenic effect of the Killevir preparation substance and medicinal form high doses can be regarded as a manifestation of the animal individual sensitivity.

A substance and the both medicinal forms of the Killevir preparation did not form a hypersensitivity of the immediate or delayed types in mice and guinea-pigs in the recommended for the clinical tests therapeutic doses.