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Report
Study of the Killevir preparation substance and two medicinal form generally-toxic effect

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Killevir is a new medicinal antiherpetic preparation on a basis of a fullerenepolyaminocaproic acid (FPACA).

The aim of the present work is a preclinical assessment of the new medicinal preparation killevir substance and two medicinal form generally-toxic effect.

A study of the acute and chronic toxicity at the preparation infusion into the laboratory animal organism by ways, which are presumed for a clinical study, was the task.

In the acute experiments on rats and rabbits, which have been once intravenously infused with an injectional medicinal form, no toxic effect of the killevir substance and medicinal forms in doses up to the 10-fold equitherapeutic ones is revealed according to the clinical signs, integral criteria and the pathomorphologic investigation results.

It is established in experiments on rats at a 7-day course of the injectional medicinal form intravenous infusion in the 1- and 5-fold equitherapeutic doses, and rabbits in the 1- and 10-fold equitherapeutic doses, that the killevir substance and preparation cause the reversible alterations of a leukocytic formula in rats due to the segmentonuclear leukocyte percent increase and the lymphocyte percent decrease. These alterations are within the physiologic norm limits, reversible after the preparation abolition and connected either with a FPACA specific activity, on which its therapeutic effect is based, or the animal organism reaction to a FPACA infusion.

A preparation and component of a dimethylsulfoxide (DMSO) preparative form in rabbits cause an insignificant reversible influence on a functional state of the liver – a decrease of a general protein concentration in the liver within the physiologic norm limits. The observed effect is mostly stipulated by a DMSO effect.

It is established on rats at a 6-month course of a substance rectal infusion in the 1-, 3- and 10-fold equitherapeutic doses, that a substance in rats causes the reversible alterations of a leukocytic formula due to an increase of the segmentonuclear leukocyte percent and a decrease of the lymphocyte percent. These alterations are within the physiologic norm abolition. A substance in rats influences a leucopoiesis: an appearance of the neutrophile young forms – the neutrophilic metamyelocytes is noted in a part of the animals in the peripheral blood smears at a dose of 1,4 mg/kg of rats. The medicinal form is normalized after the preparation abolition.

It is established on rabbits at a 6-month course of the killevir suppository medicinal form infusion in the 1- and 10-fold equitherapeutic doses, that the

preparation influences a leucopoiesis in rabbits: an appearance of the single (1-2%) young forms of neutrophiles – the neutrophilic metamyelocytes and myelocytes is noted in the peripheral blood smears of rabbits, receiving the preparation in a dose of 280 mg/kg after the infusion course termination.

A locally-irritating effect of the killevir two medicinal forms is not revealed in the acute and chronic experiments in the 1-10-fold equitherapeutic doses.

The killevir preparation substance and its medicinal forms do not cause the expressed damaging effect on the organism organs and systems in a range of doses from the equitherapeutic one to the 10-times its receiving.

On a basis of the conducted investigations it can be concluded, that the killevir substance, injectional and suppository medicinal forms possess of a large therapeutic width and are safe at a durative use.

The preparation can be recommended for the clinical investigation conduction.

CONTENTS

INTRODUCTION

Killevir is a new antiherpetic preparation on a basis of the new substance – a fullerenepolyaminocaproic acid (FPACA), elaborated by the "DESCO" joint-stock company.

A FPACA is insoluble in water, soluble in a dimethylsulfoxide (DMSO).

The killevir is released in a form of the two medicinal forms: a concentrated solution for injections and the suppositoria for a rectal infusion. A concentrate before infusion is diluted with water for injections to a FPACA concentration of 1,0 mg/ml and singularly intravenously infused in a therapeutic dose of 0,7 mg/kg. The suppositoria are used by a daily course during 3 and more months at a FPACA therapeutic dose of 0,14 mg/kg.

The aim of the present work is a preclinical assessment of the killevir preparation substance and two medicinal form generally-toxic effect.

A study of the substance and preparation acute and chronic toxicity at infusion into the laboratory animal organism by ways, which are presumed for infusion into a human, was the task.

The investigations are conducted according to the "Methodical instructions on the pharmacologic substance generally-toxic effect study", M., 1997.

A FPACA solubility in water, a necessity of the FPACA presumed concentration use in the toxicologic experiments in the investigating medicinal forms of the preparation and the admissible volumes of fluids for the intravenous and rectal infusion into the animals were the limiting factors at a selection of doses for the acute and chronic toxicity study. The maximum doses of the killevir substance and both medicinal forms, which can be intravenously and rectally infused into the animals, do not exceed the 10-fold equitherapeutic doses. A duration of the FPACA and two medicinal form of the preparation course of infusion into the animals at a chronic toxicity study was established considering the killevir presumed use in a human.

1. MATERIALS AND METHODS

The following composition samples were presented for investigation by the "Desco" joint-stock company:

- a concentrate for injections: a FPACA – 50 mg; a DMSO – 1 ml and a water for injections – 2 ml;
- a DMSO "Dimexide" of the "Rospharm" Ltd. production;
- the suppositoria rectal (a candle mass): a FPACA – 10 mg, a DMSO – 200 mg and a lipophilic suppository tela Vitepsol – up to 2 g;
- a placebo (a candle mass): a DMSO – 200 mg and a lipophilic suppository tela Vitepsol – up to 2 g.

The experiments were conducted on the white outbreeding rats, received from the Central nursery of the RAMS laboratory animals (department "Kryukovo"), and on the rabbits of the shinshilla breed, received from a nursery

“Manikhino” of the veterinary preparation All-Russian state Scientific Institute. A maintenance of the animals corresponded to the sanitary rules on arrangement, equipping and maintenance of the experimentally-biological clinics (vivaria), approved by the USSR MPH on 73.07.06. The animals were fed with the granulated feeds according to the norms, approved by the USSR MPH order №755 of 77.08.12. The animals have passed a quarantine and adaptation in a condition of vivarium during 14 days.

The experimental groups of animals were formed by a method of the random selections considering a body mass as a leading value. A daily supervision of the animal general clinical state, a consumption of the feed and water was made. The animals were weekly weighed.

A solution for an intravenous infusion was daily prepared directly before use in the aseptic conditions by the killevir concentrate dilution with a water for injections. A candle mass of the killevir was melted on a water bath at a temperature of 37°C and rectally infused into the animals with a syringe and needle with a stannic olive at the end.

An acute toxicity of the substance (injectional medicinal form of the killevir) was studied on rats (by 6 males and 6 females to a dose) and rabbits (by 4 males and 4 females) at a rectal infusion in the 1, 3 and 10-fold equitherapeutic doses. A placebo, corresponding to a volume of the preparation maximum dose, was infused into the control animals.

In the acute experiments, the animals have been observed during 14 days. In a period of observation, a general state and clinical signs of the animal intoxication were assessed. On a termination of the observation date, the animals from a maximum dose and control were killed and a pathologoanatomic investigation was made.

A chronic toxicity of the killevir injectional medicinal form substance at an intravenous infusion was studied on rats (by 12 males and 12 females) in the 1- and 5-fold equitherapeutic doses. A DMSO aqueous solution in a volume, corresponding to a volume of a substance maximum dose, was infused into the control animals. There were 2 controls in an experiment on rabbits: a water for injections was infused into one control group of animals and a DMSO aqueous solution was infused into the other one. A course of infusion has been daily conducted during 7 days. A reducing period duration was 14 days.

A chronic toxicity of a substance at a rectal infusion was studied on rats (by 10 males and 10 females) in the 1, 3 and 10-fold equitherapeutic doses. A substance solution was daily prepared from the killevir concentrate for injections directly before use. A DMSO aqueous solution in a volume, corresponding to a volume of a substance maximum dose, was infused into the control animals. A course of infusion has been daily conducted by 5 days a week during 6 months. A reducing period duration was a month.

A chronic toxicity of the killevir suppository medicinal form at a rectal infusion was studied on rabbits (by 12 females) in the 1 and 10-fold equitherapeutic doses. A placebo in a volume, corresponding to a volume of the preparation maximum dose, was infused into the control animals. A course of

infusion has been daily conducted during 6 months. A reducing period duration was a month.

In the chronic experiments in 24 hours after the infusion course and reducing period termination, a half of the animals from each group was excluded from experiment for conduction of the hematologic, biochemical and pathomorphologic investigations. A material for investigation was taken in 3 dates at a study of the killevir suppository medicinal form chronic toxicity on rabbits: in 1, 6 months of infusion and after a reducing period. An eutanasia of the rats was made with a CO_2 inhalation and an eutanasia of the rabbits was made with an intracardiac injection of a potassium chloride after a preliminary anesthesia with a romitar. An autopsy has been made during an hour after the animal euthanasia.

A locally-irritating effect of the killevir medicinal forms at the intravenous and rectal infusions was assessed visually and according to the results of a histologic study of tissues, adjoining the preparation infusion site.

A hematologic analysis was made with an aid of a semiautomatic two-channel conductometric counter of cells the "Hemascreen 13" (Hospitex Diagnostics, Italy). The following parameters of blood were detected: a quantity of erythrocytes, leukocytes and thrombocytes, a hemoglobin concentration, a hematocrit, an average volume of an erythrocyte, an average content of a hemoglobin and an average concentration of a hemoglobin in an erythrocyte. A leukocytic formula of blood and a general quantity of reticulocytes were detected in a stained smear by a luminous microscopy method [2]. The smears were stained by the Romanovsky-Gimza method for calculation of a leukocytic formula of blood. The reticulocytes were stained directly in a test-tube with a brilliantcreasiol blue. The erythrocyte content was detected at the Panchenkov's apparatus, a time of the blood coagulation was detected by the Lee-White method.

The biochemical values of the blood serum were detected with an aid of a semiautomatic analyzer the "Screen Master 3000" (Hospitex Diagnostics, Italy) with a use of the following unified methods: a concentration of a general protein – by a biuretic method, a concentration of glucose and a general cholesterol – by an enzymatic colorimetric method, a concentration of urea – by an ureasic/phenol-hypochloritic method, a concentration of a creatinine – by the Yaffe's method with a deproteinization, a concentration of a general bilirubin – by the Endrassik-Groff method, an activity of the AlAT and AsAT – by the Writeman-Frenkel method, an activity of the alkaline phosphatase – by the unified kinetic method.

The urine values were detected:

- in rats and rabbits at a chronic intravenous infusion – with an aid of a semiautomatic analyzer the "Screen Master 3000" (Hospitex Diagnostics, Italy) with a use of the following unified methods: a concentration of a general protein- by a biuretic method; a concentration of urea – by an ureasic/phenol-hypochloritic method; the glucose, nitrites, ketonic bodies, a quantity of erythrocytes, a hemoglobin and pH were detected with an aid of the diagnostic stripes for investigation of urine of the "Pliva-Lachema" (Czech Republic) production.

- In rats and rabbits at a chronic rectal infusion – with a use of the test-stripes at a semiautomatic analyzer of urine the “Urisys 1100” (Roche Diagnostics, Hungary).

A morphological state of the animal visceral organs was detected visually at an autopsy and by a study of the histologic preparations. The pieces of lungs, liver, spleen, kidney, stomach, small intestine, rectum, tissues from the infusion sites and the testes in males were selected for a histologic investigation.

The pieces of the animal organs were fixed in a 10% solution of a formalin, after a standard conduction through the alcohols they were covered with a paraffin and the sections of the 5-6 mkm width were prepared. The sections were stained with a hematoxilin-eosin and put into a Canadian balm. An analysis of the histologic preparations was made under a luminous microscope.

A statistical treatment of the received results of investigations was made by the methods of a variational statistics according to the Student’s criterium.

2. RESULTS OF INVESTIGATIONS

2.1. Acute toxicity of the killevir substance and injectional medicinal form at an intravenous infusion

As a FPACA is insoluble in water and soluble in a DMSO, it is possible to receive a true solution of a substance, appropriate for intravenous infusion into the animals, only after the initial dissolution of a FPACA in a DMSO and a subsequent dilution with water. A DMSO concentration in the received solution must not exceed 3%, considering its ability to potentiate a biological effect of the many substances.

A substance solution with such parameters is received at a dilution with a water for injections of the killevir injectional concentrate in a recommended for use concentration. A use of a DMSO aqueous solution as a solvent control permits to reveal the potential side effects oa a FPACA at the background of a solvent and simultaneously to control a manipulation. Thus, the presented in divisions 2.1 and 2.2 investigations equally characterize a toxicity for the laboratory animals of both the substance itself and the killevir injectional preparative form.

An acute toxicity at an intravenous infusion was detected on the white breedless rats and rabbits of the shinshilla breed, which were receiving a single infusion of the killevir injectional solution in the FPACA doses of 0,7, 2,1 and 7,0 mg/kg. The control animals were receiving a DMSO aqueous solution in the same volume, as at a maximum dose of the preparation.

The clinical signs of the animal intoxication were absent at all the doses directly after infusion. In a period of 14 days there were no deaths, a general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, Tables 1, 2).

On termination of the observation date, the experimental animals, receiving a maximum dose of the preparation, and the control ones were killed and a pathologoanatomic investigation was made. No pathologic alterations, connected

with the preparation effect, were established at a macroscopic picture analysis of the thoracic and abdominal cavity organs.

2.2. Chronic toxicity of the killevir substance and injectional medicinal form at an intravenous infusion

2.2.1. Chronic toxicity for rats at an intravenous infusion

A chronic toxicity at an intravenous infusion was studied on the white breedless rats, into which the killevir injectional solution has been daily infused during 7 days in doses of 700 and 3500 mg/kg/day (0,7 and 3,5 mg/kg/day of a FPACA). A DMSO aqueous solution was infused into the control animals in the same volume, as at a maximum dose of the preparation. A duration of a reducing period was 14 days.

The clinical signs of poisoning and the animal death were absent at all the doses in a period of the preparation infusion and in a reducing period. A general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, Table 3).

The samples of the peripheral and venous blood, urine were selected in an equal quantity of animals from each group after a termination of the infusion course and a reducing period, after that they underwent an eutanasia (a CO_2 inhalation) and a pathologoanatomic autopsy was made.

The results of the clinical analyses and pathologoanatomic investigations are presented in the Appendix, tables №4-17.

The results of the rat peripheral blood hematologic analysis after a termination of the infusion course have demonstrated a trustworthy increase of the segmentonuclear leukocyte share ay a maximum dose in males (Appendix, table 4), and in females – a trustworthy increase of the segmentonuclear leukocytes and the lymphocyte decrease (Appendix, table 5). There were no trustworthy differences between the values of the experimental and control group animals after a reducing period (Appendix, tables 6, 7).

The observed alterations of a leukocytic formula in the males and females, receiving a maximum dose, are evidently a specific reaction of the animal organism to the preparation infusion.

The results of the rat blood serum biochemical analysis after a termination of the infusion course have demonstrated a trustworthy increase of a glucose concentration in males of the both experimental groups and an urea concentration in males at a maximum dose, compared to the control (Appendix, table 8). A general bilirubin concentration and a general protein concentration at a maximum dose are trustworthy decreased in females of the both experimental groups (Appendix, table 9). No trustworthy differences between the values of the experimental and control group animals were revealed after a reducing period (Appendix, tables 10, 11).

All the statistically trustworthy alterations, relating to control, are within the physiologic norm limits for the given type of animals. An analysis of the rat blood serum biochemical values after the infusion course and a reducing period demonstrates, that the received alterations of the several values in the experimental

group animals, are within the physiologic norm limits, sporadical and, consequently, are caused by the random reasons and not connected with the preparation effect.

According to results of the rat urine clinical analyses, there are no trustworthy differences between the values of the experimental and control group animals after a termination of the infusion course and a reducing period (Appendix, tables 12-15).

A pathologoanatomic investigation of rats was made after a 7-day course of an intravenous infusion and after two weeks of a reducing period. The lungs, liver, spleen, kidney, small intestine, a tissue from the infusion site and testes were taken for a histologic investigation.

After a course of the killevir preparation intravenous injections, a hair integument was smooth, shining, a skin was elastic, movable, a subcutaneous fat was moderately expressed at an external examination of the animal cadavers of all the experimental and control groups. An anatomically right location of the visceral organs was observed at an autopsy of the thoracic and abdominal cavities.

An exterior and state of the organs had an equal picture in all the investigating groups.

The lungs are without pathologic alterations, a normal lobar structure was detected, the lobes are of a pale-pink color, a sanguineofoamy fluid is on a section.

The liver is not augmented, a capsule is smooth, shining, of a dark-brown color, it is plethoric on a section.

The spleen is of a stretched form, purple color, it is soft and crumbly according to a consistency with an abundant scrape on a section.

The kidneys are symmetrically located, of a dark-brown color, they had a bean-like form, a fibrous capsule is shining, easily removable.

A small intestine is filled with contents with a small quantity of mucus, without the signs of inflation, a mucous membrane is without ulcerations.

A site of infusion – the traces of injections into the caudal vein are observed.

The testes are of an oblongatal form, pale-grey color, a paste-like consistency. A microscopic structure of the animal organs after a course of the killevir preparation intravenous injections in doses of 0,7 mg/kg and 3,5 mg/kg did not differ from a structure oa the control group rat organs and totally corresponded to the following description.

The lungs – the bronchi, bronchioles, alveoles had a normal structure, the interalveolar septa were in places enlarged, the lymphoid cell swarms were noted along the large and middle bronchi.

The liver – the central veins and the portal tract veins contained the regular elements of blood, the hepatocytes were without the signs of dystrophy, the Kupffer cells were without peculiarities.

The spleen – a thin capsule with the sparse, moving to parenchyma trabecules, a red pulp is situated with a large quantity of the regular elements of blood, a white pulp is mainly presented by the lymphocytes and macrophages.

The kidneys had a thin capsule, the capillaries of the cortical substance vascular glomeruli are plethoric, the epithelium of the proximal and distal canals is well differentiated, the canal lumens are without peculiarities.

A small intestine is covered with fibres, covered with the epithelial and goblet cells, there is a lymphohistiocytic infiltration in a submucous layer of intestine.

A site of infusion – a caudal vein in a site of infusion has the signs of regeneration after a traumatic damage with an injectional needle; the remote areas of vein are not damaged.

The testes – all the stages of a spermatogenesis are present in the majority of canals, a number of canals with a shelled epithelium is insignificant.

A series of the meaningful alterations is noted at an analysis of the rat visceral organ mass coefficient meanings after a course of the killevir preparation intravenous infusion (Appendix, table 16).

Thus, a mass of the heart is statistically trustworthy increased in males, receiving the killevir in a dose of 3,5 mg/kg/day. The liver mass in the both experimental groups as well as the spleen mass at a dose of 0,7 mg/kg are trustworthy increased in females. The received meanings can testify to the given organ plethora as a result of an excessive blood filling because of the errors at euthanasia, considering a lack of the structural alterations in organs.

No differences in the macro and microscopic structure of the visceral organs were noted after a reducing period in rats of the experimental and control groups, the mass coefficients of the organs did not substantially differ (Appendix, table 17).

Thus, as a result of the conducted pathomorphologic investigation, no signs of the killevir preparation damaging effect on the experimental rat organism were established at an intravenous infusion in doses up to the 5-fold equitherapeutic one.

2.2.2. Chronic toxicity for rabbits at an intravenous infusion

A chronic toxicity at an intravenous infusion was detected on the rabbit females, into which the killevir injectional solution has been multiply infused during 7 days in doses of 700 and 7000 mg/kg/day (0,7 and 7,0 mg/kg/day of a FPACA). The two control groups of animals were formed in the given investigation: a water for injections was infused into one control group of animals, a DMSO aqueous solution was infused into the other one in the same volume, as at a maximum dose of the preparation. A duration of a reducing period was 14 days.

The clinical signs of the animal poisoning were absent at all the doses in a period of the preparation infusion and in a reducing period. A general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, table 18).

A trustworthy decrease of a hemoglobin quantity, a general quantity of the erythrocytes, a decrease of the blood coagulation time and an increase of the thrombocytes general quantity, compared to the hematologic values of the control with water, are noted at a dose of 7,0 mg/kg/day after the killevir 7-fold

intravenous infusion in rabbits (Appendix, table 19). A trustworthy increase of the leukocyte general quantity, compared to the control with water, is noted in a control with a DMSO and at a dose of 0,7 mg/kg/day. The pointed alterations do not exceed the physiologic norm for the rabbits.

Only a trustworthy increase of the thrombocytes general quantity is established after a reducing period in the experimental group of rabbits, receiving the preparation in a dose of 7,0 mg/kg/day., in comparison with the hematologic values of the control groups, which, however, does not exceed a physiologic norm (Appendix, table 20). All the other hematologic values have no differences compared to the hematologic values of the control groups.

The results of the rabbit blood serum biochemical analysis after a termination of the infusion course have demonstrated a statistically trustworthy decrease of a general protein concentration in a control with a DMSO and in animals of the both experimental groups, compared to the control with water (Appendix, table 21). The 3,5-times statistically trustworthy exceeding of the ALT activity, relating to the control with water, is also established in a control group, receiving a DMSO, while the ALT activity is ~2-times exceeding in animals of the experimental groups (statistically is unreliable). A statistically trustworthy decrease of a cholesterol level and a decrease of the alkaline phosphate activity are established at a dose of 0,7 mg/kg/day. The statistically trustworthy alterations, relating to the control, are within the physiologic norm limits for the given type of animals.

There are the trustworthy differences between the values of the experimental and control group animals after a reducing period (Appendix, table 22).

An analysis of the given biochemical investigations demonstrates, that only a decrease of a general protein concentration can be connected with the preparation infusion. The observed effect is mainly stipulated by a DMSO effect. An influence on the animal liver functional state is insignificant and reversible.

No trustworthy differences between the values of the experimental and control group animals are revealed, according to the results of the rabbit urine biochemical analysis after the preparation infusion and a reducing period (Appendix, table 23-24).

A pathologoanatomic investigation of rabbits was made after a 7-day course of the killevir preparation intravenous infusion and after two weeks of a reducing period. By five rabbits were investigated in each experimental and control groups. The pieces of the lung, liver, spleen, kidney and ear in the infusion site and at a distance of 1 cm from it in a direction of the ear basis were taken for a histologic investigation.

At an external examination of the animals: a hair integument is integral, a skin is elastic, movable, a subcutaneous fat is moderately expressed. An anatomically correct location of the visceral organs was noted at an autopsy of the thoracic and abdominal groups, the animal visceral organs had no the signs of damage or the deviations from the anatomic norm.

A microscopic structure of the rabbit organs in the control and experimental groups had no any signs of pathology. The vein below the infusion site is without the signs of damage, the endothelium is not changed.

The statistically meaningful differences of the liver mass coefficients at a dose of 7,0 mg/kg/day and the kidney at a DMSO infusion are established according to the morphometry results (Appendix, table 25). It can be presumed, that the given increase of the organ mass took place as a result of the excessive blood filling because of an euthanasia, considering a lack of the structural alterations in the liver and kidneys, as well as the fact, that the individual meanings of the organ mass are within the anatomic norm limits for the given type of animals.

No differences in the macro- and microscopic structure of the control and experimental group rabbit organs are revealed after a reducing period. The statistically meaningful differences of the organ mass coefficients were also absent (Appendix, table 26).

Thus, the results of a pathomorphologic investigation have not revealed the killevir preparation damaging effect on the organs and tissues at an intravenous infusion into the rabbits during 7 days in doses of up to the 10-fold equitherapeutic one.

2.3. Acute toxicity of the killevir substance and suppository medicinal form at a rectal infusion

An acute toxicity of a substance at a rectal infusion was studied on rats and rabbits, into which a FPACA solution was once infused (it was prepared from the killevir concentrate for injections) in doses of 140, 420 and 1400 mg/kg (0,14, 0,42 and 1,4 mg/kg of a FPACA). A DMSO aqueous solution was infused into the control animals in the same volume, as at a maximum dose of the preparation.

The clinical signs of the animal poisoning were absent at all the doses directly after infusion. In a period of observation there were no deaths, a general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, tables 27, 28).

No control pathologic alterations of the thoracic and abdominal cavity organs, connected with the preparation effect were established at an autopsy on a termination of the observation date in animals, into which the preparation maximum dose was infused.

An acute toxicity of the suppositoria at a rectal infusion was detected on rats and rabbits, into which the killevir candle mass was once infused in doses of 28, 84 and 280 mg/kg (0,14, 0,42 and 1,4 mg/kg of a FPACA). A placebo was infused into the control animals in the same volume, as at a maximum dose of the preparation. The animals have been observed during 14 days.

The clinical signs of the animal poisoning were absent at all the doses directly after infusion. In a period of observation there were no deaths, a general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, tables 29, 30).

No pathologic alterations of the thoracic and abdominal cavity organs, connected with the preparation effect, were established at an autopsy on a termination of the observation date of animals, into which the preparation maximum dose was infused, and the control ones.

2.4. Chronic toxicity of the killevir substance and suppository medicinal form at a rectal infusion

2.4.1. Chronic toxicity of a substance for rats at a rectal infusion

A chronic toxicity of the killevir substance at a rectal infusion was studied on rats, into which a FPACA solution was multiply infused (it was prepared from the killevir concentrate for injections) in doses of 140, 420 and 1400 mg/kg/day (0,14, 0,42 and 1,4 mg/kg/day of a FPACA). A DMSO aqueous solution was infused into the control animals in the same volume, as at a maximum dose of the preparation. A course of infusion has been daily during 6 months. A duration of a reducing period was a month.

A general state of a majority of the experimental and control animals did not differ at all the doses in a period of infusion and in a reducing period. The animals were readily taking feed, evenly gaining weight (Appendix, tables 31 and 32). The differences in a dynamics of the control and experimental group animal average body mass were not revealed.

However, 6 animals have died and 2 animals have been forced to die with the similar clinical signs during an experiment in the different dates: a gradual decrease of a body mass, an alteration of a hair integument (a hair is dull and dirty, a pyloerection), a general activity decrease, a cyanosis of the mouth and eye mucous membranes, a diarrhea 1-2 weeks before a death. A death of the animals took place in the different dates from the experiment beginning:

- in the control, 1 female died at the 31st day of experiment and 1 male died at the 117th day of experiment;
- at a dose of 0,14 mg/kg/day, 2 females were killed (at the 86 and 89 days of experiment) and 3 males died (2 at the 135 and 1 at the 148 days of experiment);
- at a dose of 0,42 mg/kg/day, 1 male died at the 108 day of experiment.

The following macroscopic alterations were noted in the 2 killed females (at a dose of 0,14 mg/kg). We have found by one nodosal pyo-necrotic focus of a round form of 4,0×1,5 cm and 4,0×3,0 cm in the abdominal cavity of each of the rats in an area of a small intestine with a development of an adhesive process in the intestinal loops. At the histologic preparations they were presented in a form of a swarm of the leukocytes, macrophages, lymphoid cells, surrounded by an expressed torus of the connective cells and fibroblasts.

As a result of the postmortem alterations in the visceral organs, it was not possible to set a pathologoanatomic diagnosis of a reason of death in the died animals.

A death was mainly noted in the groups of animals, receiving a minimum dose of a FPACA, considering the fact, that the animals did not die at a maximum dose, the death of rats is not objectively connected with the killevir effect.

The samples of the peripheral and venous blood, urine were selected in a half of the animals from each group after a termination of the infusion course and a reducing period, then they underwent an eutanasia and a pathologoanatomic autopsy was made.

The results of the clinical analyses and pathologoanatomic investigations of rats are presented in Appendix, tables 33-44.

A trustworthy increase of the segmentonuclear neutrophile quantity is established at the hematologic value analysis after a 6-month course of infusion in the males and females at a dose of 1,4 mg/kg/day (Appendix, tables 33, 34), a tendency of a dose dependence according to that value is observed. An appearance of the young forms of neutrophiles – the neutrophilic metamyelocytes is noted in a part of the animals at a study of the cell morphology in the smears of the rat peripheral blood at a dose of 1,4 mg/kg/day: in 3 of 5 females (1-2%) and in 3 of 5 males (1-4%), while the young neutrophiles are not found in the control.

There were no trustworthy alterations, relating to the control group of animals, according to the other hematologic values of the rat females and males, receiving a substance in doses of 0,14 mg/kg, 0,42 mg/kg and 1,4 mg/kg.

No trustworthy alterations between the hematologic values of the experimental and control group animals are established after a reducing period (Appendix, tables 35, 36).

An increase of the segmentonuclear neutrophile general quantity in the experimental rat males and females and an appearance of the young forms of neutrophiles is apparently a response reaction of the organism to the killevir infusion.

The results of the rat blood serum biochemical analysis after a termination of the infusion course have demonstrated a statistically trustworthy decrease of a general protein concentration in males (a reverse dose dependence) and an increase of the urea (a direct dose dependence) (Appendix, table 37). The statistically trustworthy differences of the studied biochemical values of blood in the rat females are not established (Appendix, table 38).

A statistically trustworthy increase of a glucose concentration (a direct dose dependence) is established in the males at a study of the blood serum biochemical analyses, made after a reducing period (Appendix, table 39). All the studied biochemical values of the blood serum in the experimental females are at a similar level with the control (Appendix, table 40).

All the statistically trustworthy alterations of the blood serum biochemical analyses in the experimental animals, relating to the control in both dates of examination are within the physiologic norm limits for the given type of animals. An analysis of the rat blood serum biochemical values after a course of infusion and a reducing period demonstrates, that the received alterations of the several values in the experimental group animals, compared to the control animals, are within the physiologic norm limits, sporadical and, consequently, are caused by the random reasons and are not connected with the preparation effect.

There were no statistically trustworthy differences between the values of the experimental and control group animals at a study of the urine clinical analyses,

made after a 6-month course of infusion and a reducing period (Appendix, tables 41, 42).

A pathologoanatomic autopsy of the rats was made after a 6-month course of infusion and a reducing period. The pieces of the lung, liver, spleen, kidney, small intestine, rectum and a testis were taken for a histologic investigation.

At an external examination of the experimental and control group animal corpses, a hair integument was smooth, shining, a skin was elastic, movable, a subcutaneous fat was moderately expressed. An anatomically regular location of the visceral organs was noted at an autopsy of the thoracic and abdominal cavities.

An exterior and state of the organs had a similar picture without the signs of pathology in all the survived rats, in all the investigating groups.

A microscopic structure of the animal organs after the killevir preparation rectal infusion in all the doses did not differ from a structure of the control group rat organs and corresponded to the following description.

The lungs – the bronchi, bronchioles, alveoles had a normal structure, the interalveolar septa were in places enlarged, the lymphoid cell swarms were observed along the large and middle bronchi.

The liver – the central veins and veins of the portal tracts are plethoric, the hepatocytes are without the signs of dystrophy, the Kupffer cells are without peculiarities.

The spleen – a capsule is thin with the sparse, stretching to parenchyma trabecules, a red pulp is saturated with a large quantity of the regular elements of blood, a white pulp is mainly presented by the lymphocytes and macrophages.

The kidneys had a thin capsule, the capillaries of the cortical substance vascular glomeruli are plethoric, the epithelium of the proximal and distal canals is well differentiated, the canal lumens are without peculiarities.

A small intestine is covered with the fibres, covered with the epithelial and goblet cells, the lymphoid cell swarms were detected under a mucous membrane.

The rectum – the epithelium is not changed, a secretion is moderate.

The testes are without the signs of damage, there are all stages of a spermatogenesis in the spermatic canals.

A statistically trustworthy increase of the spleen mass, compared to the control, was revealed at analysis of the rat visceral organ mass coefficient meanings after a course of the killevir infusion in a dose of 1,4 mg/kg/day in males (Appendix, table 43). However, the absolute meanings of the organ mass in the separate animals are within the norm.

No differences in the visceral organ macro- and microscopic structure were noted after a period of the preparation abolition in the experimental and control group rats. A statistically trustworthy increase of the spleen and kidney mass in males (Appendix, table 44), receiving the killevir in a dose of 1,4 mg/kg/day, is established at an analysis of the visceral organ mass coefficients, compared to the control. However, the absolute meanings of the organ mass in the separate animals are within the normal limits.

As a result of the conducted histopathologic investigation of the rat visceral organs, no signs of a damaging effect on the experimental animal organism in the

FPACA doses of up to the 10-fold equitherapeutic one at the rectal infusion course of 6 months have been established.

2.4.2. Chronic toxicity of a suppository medicinal form for rabbits at a rectal infusion

A chronic toxicity of the killevir suppository medicinal form at a rectal infusion was studied on rabbits, into which a candle mass of the preparation of 28 and 280 mg/kg/day (0,14 and 1,4 mg/kg/day of a FPACA) was multiply infused. A placebo was infused into the control animals in the same volume, as at a maximum dose of the preparation. A course of infusion was daily during 6 months. A duration of a reducing period was a month.

In a period of the preparation infusion and a reducing period, the clinical signs of poisoning and the animal death at the both doses were absent, a general state of the experimental and control group animals did not differ. The animals were readily taking feed, evenly gaining weight (Appendix, table 45).

The samples of the peripheral and venous blood, urine were selected in an equal quantity of animals from each group after a month of infusion, a termination of a 6-month course of infusion and a reducing period, after that the animals underwent an eutanasia and a pathologoanatomic autopsy was made.

The results of the clinical analyses and pathologoanatomic investigations of the experimental rats are presented in Appendix, tables 46-57.

At an analysis of the rabbit hematologic values in the 3 dates of examination, a statistically trustworthy difference with the control only in one case is revealed: an increase of the eosinophile general quantity at the killevir dose of 280 mg/kg after the first month of the preparation infusion (Appendix, tables 46-48). Besides, the eosinophile quantity does not exceed a physiologic norm for the given type of animals. No trustworthy alterations are revealed according to the other hematologic values of the experimental rabbits, compared to the control animals.

It should be noted, that an appearance of the singular (1-2%) young forms of neutrophiles – the neutrophilic metamyelocytes and myelocytes (in 4 of 4 rabbits) is observed after a termination of the infusion course at a study of the cell morphology in the smears of the rabbit peripheral blood, receiving the preparation in a dose of 280 mg/kg/day. The young neutrophiles are not revealed in the control and at a dose of 28 mg/kg/day. The young forms of neutrophiles in the rabbit blood of all the groups are not found after a reducing period.

The young neutrophile exit to the peripheral blood is apparently the rabbit organism reaction to the preparation infusion.

No statistically trustworthy alteration, relating to the control in the experimental animals, are revealed at a study of the blood serum biochemical analyses (Appendix, tables 49-51) and the clinical analyses of urine (Appendix, tables 42-54), made in 3 dates of examination.

A pathologoanatomic autopsy of the rabbits was made in 1 and 6 months of the killevir preparation rectal infusion and after a reducing period. By four rabbits were investigated in each experimental and control groups. The pieces of the lung, liver, spleen, kidney and rectum were taken for a histologic investigation.

At an autopsy and external examination, a fur integument in all the animals was smooth, a skin was elastic, movable, a subcutaneous fat was moderately expressed. An anatomically regular location of the visceral organs was noted at an autopsy of the thoracic and abdominal cavities.

In all the investigating groups, an exterior and state of the organs had a similar picture without the signs of pathology.

A macroscopic structure of the animal organs in a month after the killevir preparation infusion had a similar structure in the control and experimental groups without the signs of pathology, connected with the preparation effect and totally corresponded to the following description.

The lungs – the bronchi and bronchioles had a normal structure on sections, the interalveolar septa were in places enlarged due to the erythrocytes, the lymphoid cell swarms were noted along the large and middle bronchi.

The liver – the intralobular capillary network is well developed, the hepatocytes are of a polygonal form, the Kupffer cells are without peculiarities.

The spleen – a capsule is thin with the sparse, stretching to parenchyma trabecules, a red pulp is saturated with the regular elements of blood, a white pulp is mainly presented by the lymphocytes and macrophages.

The kidneys - the capillaries of the cortical substance vascular glomeruli are plethoric, the epithelium of the proximal and distal canals is not changed, the canal lumens are free.

The rectum – the epithelium is without the signs of damage, a secretion is moderate.

A statistically meaningful increase of the liver value in the animals, receiving the killevir in a dose of 28 mg/kg/day during a month is established at an analysis of the visceral organ mass coefficients (Appendix, table 55). At the same time, the absolute meanings of the liver mass in the separate animals are within the limits of the anatomic norm values, besides, the effect dose dependence is absent.

No differences in the animal visceral organ mass coefficients are established after a termination of a 6-month course of the preparation infusion (Appendix, table 56).

A statistically trustworthy increase of the liver in the animals receiving the killevir in a dose of 28 mg/kg/day is established after a reducing period at analysis of the visceral organ mass coefficients, as after a month of the preparation infusion (Appendix, table 57). And in such a case, the absolute meanings of the liver mass in the separate animals are within the limits of the anatomic norm values.

No alterations, connected with the killevir effect, are found at a microscopic investigation of the visceral organ preparations in the 3 dates of examination.

Thus, as a result of the conducted histopathologic investigation, no signs of the killevir preparation damaging effect on the experimental rabbit organism at its infusion in doses of up to the 10-fold equitherapeutic ones, with a course of up to 6 months, are established.

2.5. A locally-irritating effect of the killevir medicinal forms: at an intravenous and rectal infusion

A locally-irritating effect of the killevir two medicinal forms was assessed in the acute and chronic experiments in the 1-10-fold equitherapeutic doses.

At an intravenous infusion (1- and 7-fold course daily) of the killevir injectional medicinal form in rats and rabbits, the vein in a site of infusion has the signs of regeneration after a traumatic damage with an injectional needle; the remote from the direct site of infusion areas of the vein have no any signs of damage, the endothelium is not changed.

At a rectal infusion (1-fold and a 6-month course daily) of the killevir substance (rats) and suppository medicinal form (rabbits), no irritation of the rectum in the rats and rabbits was observed: the epithelium is without the signs of damage, a secretion is moderate.

CONCLUSION

A generally-toxic effect of the killevir preparation substance (FPACA) and two medicinal forms is studied on the white breedless rats and rabbits of the shinshilla breed in the acute and chronic experiments.

A toxic effect of the killevir substance and two medicinal forms in doses of up to the 10-fold equitherapeutic ones is not revealed in the acute experiments on rats and rabbits, into which the injectional medicinal form was once intravenously infused in doses of 0,7, 2,1 and 7,0 mg/kg (by a substance), a FPACA solution was rectally infused in doses of 0,14, 0,42 and 1,4 mg/kg (by a substance), according to the clinical signs, integral criteria and the pathomorphologic investigation results.

A chronic toxicity of the killevir injectional medicinal form has been studied at a 7-day course of an intravenous infusion on rats in doses of 700 and 3500 mg/kg/day (0,7 and 3,5 mg/kg/day by a substance) and on rabbits in doses of 700 and 7000 mg/kg/day (0,7 and 7,0 mg/kg by a substance).

According to the results of the clinical observations of the animal general state in a period of infusion and 2 weeks of a reducing period, the animal state investigations in 24 hours after a termination of the infusion course and a reducing period, it is established, that:

- the killevir substance and preparation do not cause the alterations of the animal general state, the majority of the hematologic values, the blood serum biochemical analyses and the clinical analyses of urine, do not damage a morphological structure of the experimental animal visceral organs.
- the killevir substance and preparation cause in rats the reversible alterations of a leukocytic formula due to an increase of the segmentonuclear leukocyte percent and a decrease of the lymphocyte percent. These alterations are within the physiologic norm limits, reversible after the preparation abolition and connected with either a FPACA specific activity, on which its therapeutic effect is based, or with the animal organism reaction to a FPACA infusion.
- the killevir preparation and a DMSO (a preparative form component) cause in rabbits an insignificant reversible influence on a functional state of the animal liver.
- a decrease of a general protein concentration in the liver in the physiologic norm limits. The observed effect is mainly stipulated by a DMSO effect.

A chronic toxicity of the killevir substance has been studied on rats at a 6-month course of a rectal infusion in doses of 0,14, 0,42 and 1,4 mg/kg/day.

According to the results of the clinical observations of the animal general state in a period of infusion and a month of a reducing period, the animal state investigations in 24 hours after a termination of the infusion course and a reducing period, it is established, that:

- a substance does not cause an alteration of the animal general state, the majority of hematologic values, the blood serum biochemical analyses and the clinical analyses of urine, does not damage a morphological structure of

the experimental animal visceral organs. A death of the part of the animals in different experimental groups, except for those, receiving 1,4 mg/kg/day, is not objectively connected with a FPACA effect.

- a substance causes in rats the reversible alterations of a leukocytic formula due to an increase of the segmentonuclear leukocyte percent and a decease of the lymphocyte percent. These alterations are within the physiologic norm limits, reversible after the preparation abolition and connected with either a FPACA specific activity, on which its therapeutic effect is based, or with the animal organism reaction to a FPACA infusion.
- a substance influences a leucopoiesis in rats: an appearance of the young forms of neutrophiles – the neutrophilic metamyelocytes is noted in the rat peripheral blood smears at a dose of 1,4 mg/kg in a part of the animals. A leukocytic formula is normalized after the preparation abolition.

A chronic toxicity of the killevir suppository medicinal form has been studied on rabbits at a 6-month course of a rectal infusion in doses of 28 and 280 mg/kg/day (0,14 and 1,4 mg/kg/day by a substance).

Besides, it is established, that:

- the preparation does not cause the alterations of the animal general state, the majority of hematologic values, the blood serum biochemical analyses and the clinical analyses of urine, it does not damage a morphological structure of the experimental animal visceral organs.
- the preparation influences a leucopoiesis in rabbits: an appearance of the singular (1-2%) young forms of neutrophiles.
- the neutrophilic metamyelocytes and myelocytes is noted in the rabbit peripheral blood smears, receiving the preparation in a dose of 280 mg/kg/day after a termination of the infusion course. That effect is evidently stipulated by a FPACA effect. A leukocytic formula is normalized after the preparation abolition.

In the acute and chronic experiments in the 1-10-fold equitherapeutic doses, a locally-irritating effect of the killevir two medicinal forms is not revealed.

Thus, the killevir preparation substance and its medicinal forms have no expressed damaging effect on the organism organs and systems in a range of doses of the equitherapeutic one 10-times its exceeding.

On a basis of the conducted investigations it can be concluded, that the killevir substance, injectional and suppository medicinal forms possess of a great therapeutic capacity and are safe at a durative use.

The preparation can be recommended for the clinical investigations.