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REPORT
Experimental study of the killevir preparation reproductive toxicity

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PAPER

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Key words: Killevir, fullerenepolyaminocaproic acid, reproductive toxicity, embryotoxic effect, teratogenic effect, antenatal period of development, gonadotoxic effect

The aim of the conducted investigations was an experimental assessment of the new pharmacologic preparation Killevir reproductive toxicity.

A compound is a derivative of a fullerene and aminocaproic acid – a fullerenepolyaminocaproic acid. The investigating compound is planned to be used as a medicinal preparation, with an antiherpetic activity. The two medicinal forms of the Killevir: suppositoria and injectional form are proposed for use.

The experimental investigations are conducted on the outbreeding white rats in doses up to a 10-fold equitherapeutic one. The animals, to which a suppository tela with a DMSO was rectally administered, either a DMSO aqueous solution and a water for injections (control) were intravenously administered, were selected as the control groups, because the investigating preparation contains in its tela a dimethylsulfoxide (DMSO).

The meaningful signs of a damaging effect are not established at an experimental study of the Killevir preparation reproductive toxicity in a form of suppositoria for a rectal infusion. However, the noted increase of the fetus liver blood-filling at a use of the three- and ten-fold equitherapeutic doses deserves attention.

A disturbance of a fetus general development, expressed in a decrease of the mass and sizes, including the preparation equitherapeutic dose effect, is established at a study of the Killevir injectional form.

There are all grounds to suppose, that the revealed embryotoxic effects are caused by a DMSO. A use of the preparation is connected with a risk for a fetus, considering the fact, that a DMSO is an integral part (a substance solvent) of the Killevir medicinal forms.

INTRODUCTION

The results of experimental investigations of the new original preparation Killevir reproductive toxicity, possessing of an antiviral activity, are presented in the report. The preparation authors recommend it as an antiherpetic preparation.

A substance of the Killevir is a derivative of a fullerene and aminocaproic acid – a fullerenepolyaminocaproic acid. A substance is insoluble in water, that is why a dimethylsulfoxide (DMSO) is used for its dissolution.

The two medicinal forms of the Killevir: suppositoria and injectional form are proposed for use. The main information of a composition and conditions of the medicinal form use:

Suppositoria rectal:

a fullerenepolyaminocaproic acid – 10 mg,

a DMSO - 200 mg,

a vitepsol – up to 2000 mg.

The suppositoria are designed for a daily one-fold rectal use in a dose of 1 suppository, with a course of not less than 3 months.

Concentrate for injections (to a dose):

a fullerenepolyaminocaproic acid – 50 mg,

a DMSO - 1 ml,

a water for injections – 2 ml.

The concentrate is diluted in 30 ml of water for injections before an injection. The preparation is once used by an intravenous infusion.

1. MATERIALS AND METHODS

The investigations are conducted according to the "Methodical instructions on the pharmacologic substance reproductive toxicity study"/Manual on experimental (preclinical) study of the new pharmacologic substances. – Ed. 2, revised and supplemented. – M: The "Publishing house "Medicine" joint-stock company, 2005, p. 87-100.

The outbreeding white rats, acquired in the central nursery of the laboratory animals of the Russian Academy of Medical Sciences (RAMS), department "Kryukovo", are used in the work. A study of the embryotoxic and teratogenic effect of the killevir was made in the antenatal period. A study of the Killevir gonadotoxic effect has been made during 30 days on males and during 15 days – on females. Totally, 223 rats are used in experiments on the embryotoxicity and gonadotoxicity.

A suppository mass of the killevir has been infused into the females of rats rectally in doses of 28,6 (equitherapeutic), 85,8 and 286,0 mg/kg of a body mass from the first to the twentieth day of pregnancy for the embryotoxic and teratogenic effect study. The killevir was intravenously infused into the pregnant females in doses of 0,5 (equitherapeutic); 1,5 and 5,0 ml/kg according to the following scheme:

for a study of an influence on a preimplantation period: in the 1^{st} and 3^{d} days of pregnancy;

for a study of an influence on the early period of organogenesis: in the 7^{th} and 9^{th} days of pregnancy;

for a study of an influence on the late period of organogenesis: in the 11^{th} and 13^{th} days of pregnancy;

for a study of an influence on the osseous system development: in the 16^{th} and 18^{th} days of pregnancy.

A suppository tela with a DMSO or an aqueous solution of a DMSO in a quantity and concentrations, corresponding to its content in the corresponding medicinal forms, were infused into the separate groups of animals at all the stages of investigation, because the killevir has a DMSO in its composition, possessing of the embryotoxicity. Besides, a water for injections (control) was infused into the separate groups of animals. The female behavior, clinical signs and dynamics of the body mass have been registered during the preparation infusion. On the 20^{th} day of pregnancy the animals were killed and an analysis of the fetus state was made.

The killevir in doses of 28,6 and 286,0 mg/kg and a suppository tela with a DMSO in a dose of 286,0 mg/kg have been daily infused during 30 days rectally and intravenously in doses of 0,5 and 5,0 ml/kg, and an aqueous solution of a DMSO in a dose of 5,0 ml/kg once in the first day of experiment for a study of a toxic effect on the male gonads. A water for injections was infused into the control animals.

The killevir has been rectally and daily infused during 15 days in a dose of 286,0 mg/kg for a study of a toxic effect on the female gonads. A suppository tela with a DMSO in a dose of 286,0 mg/kg was infused into the control ones.

A statistical assessment of the results was made with a use of the Student's t-criterium.

1.1. Methods of study of the killevir embryotoxic effect, registered in the antenatal period

The males were placed to females from the evening in a ratio of 1:2 for receipt of the rats with a know date of pregnancy. In the morning the native smears of the vaginal contents, which were analyzed under a microscope, were prepared. A value of the female fertilization was a presence of the spermatozoa in the smears.

At the 20 days after fertilization, the animals were killed for the preparation toxic effect study in the antenatal period of development and their autopsy was made. A number of yellow bodies in the ovaries, a number of the implantation sites in the uterus, a number of the living and resorbed fetuses were calculated in the pregnant females. On a basis of this data, a level of the pre- and postimplantation mortality of the embryos was detected.

The fetuses of each brood were carefully examined for detection of the external anomalies of development. Then the fetuses were weighed and their craniocaudal size was detected, a conclusion of the fetus maturity degree was made according to these values.

After that, a half of all the fetuses from a posterity of each rat was left for investigation of the fetus skeleton state according to the Dowson's method in modification of the embryology department of the USSR AMS SRI of Embryology, the visceral organs were investigated in the second half according to the Wilson's method in modification of the embryology department of the USSR AMS SRI.

1.2. Methods of study of the killevir toxic effect on the gonads of males and females.

The killevir and a suppository tela with a DMSO were once infused in the first day of experiment for a study of a toxic effect on the male gonads. The animals were daily examined and weighed and after that they were killed and an autopsy was made.

The testises, appendages and prostate were weighed in the animals for detection of the mass coefficients. The testis appendage was used for a suspension receipt and preparation of a smear for calculation of the spermatozoon pathologic form relative quantity. The second testis was fixed in a formalin 10% and the histologic sections were prepared for analysis of a spermatogenic epithelium, which was assessed according to the following quantitative values: a spermatogenesis index; a quantity of the normal spermatogones.

The killevir and an aqueous solution of a DMSO were infused in the 1^{st}, 4^{th} and 10^{th} days of experiment for a study of a toxic effect on the female gonads.

During 15 days the animals have been examined and weekly weighed, after that they were killed and an autopsy was made. The ovaries, which were fixed in a formalin 10% with a following preparation of the histologic selection through the whole organ with the 6 micrometre thickness and staining with a hematoxilin and eosin and the following calculation of the structural and functional elements in the ovary: the folliculi with a layer of the granulous cells; the folliculi with the two and more layers of the granulous cells; the Graafian vesicles and atretic bodies, were taken from the females. The pointed elements were calculated along the whole surface of a section.

2. RESULTS OF INVESTIGATIONS

2.1. Results of the killevir embryotoxic effect investigation at a rectal infusion

No differences in a dynamics of the animal body mass have been noted in all the experimental groups, compared to the control ones, during the whole period of pregnancy at a rectal infusion of the killevir and a suppository tela with a DMSO (table 1).

Table 1.

Dynamics of the pregnant female body mass alternations during the whole date of the killevir rectal infusion

Group	Initial weight	1st week	2nd week	3d week
Control	211,3±3,9	242,0±5,7	272,0±7,3	310,0±0,4
Killevir 28,6 mg/kg	215±2,7	246,2±3,2	271,3±4,5	316,0±10,4
Killevir 85,8 mg/kg	208,0±5,2	233,,7±5,0	266,3±6,0	309,0±9,0
Killevir286,0 mg/kg	210,6±4,5	242,5±5,1	275,9±6,8	328,4±11,7
Suppository tela with a DMSO 286,0 mg/kg	209,0±4,2	240,0±5,1	270,0±8,3	305,7±11,4

An assessment of the rat generative function state under the killevir influence has revealed a trustworthy increase of the implantation site and lliving fetus quantity in a group of animals, receiving the preparation in a dose of 85,8 mg/kg of a body mass (table 2). The alternations of the given values are not dose-dependent. An analysis of the other values has not revealed the differences between the experimental and control animals.

Table 2.

Results of a quantitative assessment of the killevir embryotoxic effect on the white rats at a rectal infusion

Values	Group

	Control	Killevir 28,6 mg/kg	Killevir 85,8 mg/kg	Killevir 286,0 mg/kg	Suppository tela with a DMSO 286,0 mg/kg
Quantity of the pregnant females	12	11	12	15	10
Results of an autopsy (counting a female)					
Yellow bodies	15,9±1,8	13,0±0,7	14,5±1,1	14,5±1,0	13,0±0,6
Implantation sites	7,2±1,2	9,5±0,6	10,3±0,5*	8,7±0,9	9,1±0,8
Living fetuses	6,4±1,2	9,0±0,7	9,6±0,6*	8,3±1,1	7,9±0,9
Resorbed fetuses	0,9±0,3	0,5±0,2	0,8±0,4	0,5±0,3	1,2±0,4
Preimplantation mortality, %	45,7±9,2	26,7±4,1	25,9±4,7	36,4±6,9	29,3±6,1
Postimplantation mortality, %	5,8±2,1	3,8±1,8	4,5±1,9	2,6±1,4	9,7±3,2
Placental and fetal coefficient, %	0,15±0,02	0,20±0,01	0,20±0,02	0,20±0,02	0,20±0,02
Parameters of the fetuses					
Body mass, g	3,9±0,5	4,5±0,1	4,4±0,2	3,9±0,4	4,3±0,2
Craniocaudal size, cm	3,8±0,5	4,2±0,1	4,4±0,1	3,8±0,4	4,3±0,1
Results of the fetus external examination					
Quantity of the examined fetuses	83	99	115	124	79
Quantity of the fetuses with the development anomalies: general / %	0/0	0/0	0/0	0/0	1/1,27
Results of the fetus osseous system state assessment					
Quantity of the examined fetuses	43	51	59	61	37
Quantity of fetuses with the development anomalies: general / %	1/2,3	0/0,0	3/5,1	8/13,1	5/13,5

Results of the fetus visceral organ state assessment					
Quantity of the examined fetuses	40	48	56	63	42
Quantity of fetuses with the development anomalies: general / %	3/7,5	1/2,1	6/10,7	9/14,3	5/11,9
Note: *p>0,05					

An external examination of the fetuses during an autopsy of the experimental group females has revealed a case of a fetus pathology in a group, receiving a suppository tela with a DMSO in a dose of 286,0 mg/kg. A general underdevelopment of a fetus as well as aplasia of the left anterior extremity and syndactylia in the both posterior extremities are noted. The fetus pathology in all the other groups is not revealed.

An analysis of the fetus visceral organ state by a method of serial sections has demonstrated a trustworthy increase of the fetus quantity with the liver vessel plethora in the groups, where the rats were rectally receiving the killevir in doses of 85,8 mg/kg and 286,0 mg/kg (table 3).

Upon the whole, an increased level of the hemorrhage appearance was observed in these groups. There were no substantial differences with the control on the other anomalies.

Table 3.

Results of investigation of the killevir toxic effect on the fetus visceral organ state at a rectal infusion

Group	Quantity of fetuses with the visceral organ anomalies (abs./%)					
	Plethora of the liver vessels	Hemorrhages in the liver	Subcutaneous hemorrhage	Hemorrhage in the kidneys	Hemorrhage in the intestine and stomach	Hemor in the mat
Control	0/0	0/0	0/0	0/0	0/0	3/7
Killevir 28,6 mg/kg	0/0	0/0	0/0	1/2,1	0/0	0/
Killevir 85,8 mg/kg	4/7,1	0/0	0/0	0/0	1/1,8	1/1
Killevir 286,0 mg/kg	4/6,3	1/1,6	2/3,2	0/0	0/0	2/3
Suppository tela with a DMSO	0/0	0/0	0/0	0/0	2/4,8	3/7

286,0 mg/kg						

A trustworthy inhibition in the breast bone ossification is revealed at a study of the osseous system development in a group of embryos, the mothers of which were rectally receiving the killevir in a dose of 286,0 mg/kg (table 4). Besides, the higher value of the separate bone ossification inhibition is noted at an effect of a suppository tela with a DMSO.

Table 4.

Results of investigation of the killevir toxic effect on the fetus osseous system development at a rectal infusion

Group	Quantity of the fetuses with the osseous system anomalies (abs. / %)			
	Inhibition of a breast bone ossification	Inhibition of a hyoid bone ossification	Inhibition of the metacarpal and metatarsal bone ossification	Lack of extremities
Control	0/0	1/2,3	0/0	0/0
Killevir 28,6 mg/kg	0/0	0/0	0/0	0/0
Killevir 85,8 mg/kg	3/5,1	0/0	0/0	0/0
Killevir 286,0 mg/kg	6/9,9	2/3,3	0/0	0/0
Suppository tela with a DMSO 286,0 mg/kg	1/2,7	2/5,4	1/2,7	1/2,7

2.2. Results of the killevir embryotoxic effect investigation at the intravenous infusion

An observation of the experimental animals at the killevir intravenous infusion has not revealed any alternation of their physical state. A dynamics of the experimental and control group rat body mass alternations was positive and had no the trustworthy differences excluding a group, receiving a DMSO aqueous solution in a maximum dose in the 16^{th} and 18^{th} days of pregnancy. The animal mass in the given group was reliably higher than the control (table 5), which was explained by a great number of fetuses in the average in each female as it has been established at an autopsy of the animals.

Table 5.

Dynamics of the rat body mass alternation at the killevir intravenous infusion

Group	Initial weight	1^{st} week	2^{nd} week	3^{d} week

Control	197,2±4,9	225,0±6,3	254,7±7,7	296,3±11,3
Preimplantation (the 1st and 3d days of infusion)				
Killevir 5,0 ml/kg	205,8±3,3	228,3±4,8	255,8±5,4	316,8±13,2
Aqueous solution of a DMSO 5,0 ml/kg	197,9±6,2	220,0±5,0	253,6±5,4	323,6±7,1
Organogenesis (the 7th and 9th days of infusion)				
Killevir 0,5 ml/kg	181,7±10,8	205,8±9,5	241,7±12,6	284,0±24,4
Killevir 1,5 ml/kg	189,3±9,9	216,4±9,8	245,0±11,2	273,0±24,1
Killevir 5,0 ml/kg	182,5±6,2	208,3±8,2	244,2±13,2	281,5±19,0
Aqueous solution of a DMSO 0,5 ml/kg	185,0±13,4	208,3±9,1	235,0±11,8	282,3±22,4
Aqueous solution of a DMSO 1,5 ml/kg	192,5±11,5	209,2±10,0	241,7±12,2	298,2±23,9
Aqueous solution of a DMSO 5,0 ml/kg	181,0±7,0	204,0±9,9	232,0±13,2	266,4±22,5
Organogenesis (the 16th and 18th days of infusion)				
Killevir 0,5 ml/kg	186,3±15,9	217,5±10,1	252,5±12,5	310,5±21,4
Killevir 1,5 ml/kg	196,3±13,8	225,0±11,7	250,0±15,2	285,3±28,0
Killevir 5,0 ml/kg	182,5±14,2	212,5±7,8	250,0±7,1	295,0±5,4
Aqueous solution of a DMSO 0,5 ml/kg	180,0±4,1	209,5±6,1	243,8±10,3	286,8±24,0
Aqueous solution of a DMSO 1,5 ml/kg	192,5±10,1	216,3±9,5	246,3±6,6	280,3±21,0
Aqueous solution of a	188,8±17,1	210,0±18,4	252,5±21,0	305,0±22,2

DMSO 5,0 ml/kg				
Ossification (the 16th and 18th days of infusion)				
Killevir 5,0 ml/kg	205,0±4,6	241,3±9,7	275,0±8,7	307,0±22,5
Aqueous solution of a DMSO 5,0 ml/kg	201,3±7,5	236,3±6,3	276,3±9,4	342,8±11,1*
Note: *p>0,05				

The results of an autopsy of the control and experimental group females have demonstrated, that such parameters of the embryotoxic effect assessment, as a quantity of yellow bodies in the ovaries and a quantity of the implantation sites, and, consequently, a preimplantation mortality of the embryos, were not statistically different (table 6). An exclusion was a group, intravenously receiving a DMSO aqueous solution in the 16th and 18th days of pregnancy in a dose of 5,0 ml/kg. A trustworthy increased quantity of the implantation sites and living fetuses is noted in the given group animals, and a preimplantation mortality is trustworthy decreased. There were no statistically meaningful differences for all the other experimental groups.

An external examination of the fetuses during the female autopsy has revealed a case of the embryo pathology in a group, intravenously receiving a DMSO aqueous solution in a dose of 5,0 ml/kg in the 1st and 3d days, and a case of the embryo pathology, causing an intrauterine death in a group, intravenously receiving a DMSO aqueous solution in a dose of 5,0 ml/kg in the 16th and 18th days of pregnancy. In the first case, a general underdevelopment, the brain hernia, as well as the upper jaw hypoplasia with the upper lip and hard palate splitting, were noted, in the second case, a lack of the hands and feet, as well as the soft tissue lysis as a result of the intrauterine death, were noted. The cases of a rude pathology (the external anomalies of development) were not noted in the groups of animals, receiving the killevir.

A trustworthy decrease of the embryo body mass and craniocaudal size, excluding the groups, into which the killevir and a DMSO aqueous solution were infused in the 16th and 18th days of pregnancy, was noted in all the experimental groups, regardless of a dose and date of the killevir or a DMSO solution infusion (table 6). It testifies to the embryo- and fetotoxic effect, i.e. an influence in the organogenesis period. There are all grounds to presume, that the given effect is caused by a DMSO. However, the preparation use will be always connected with a risk for a fetus, considering the fact, that a DMSO is an integral part (a substance solvent) of the killevir preparation injectional form.

Table 6.

Results of a quantitative assessment of the killevir embryotoxic effect on the white rats at an intravenous infusion

Results of an autopsy (counting a female)								
Group	Quantity of the pregnant females	Yellow bodies	Implantation sites	Living fetuses	Resorbed fetuses	Preimplantation mortality, %	Postimplantation mortality, %	Placental and fetal coefficient
Control	10	15,7±1,5	9,5±1,6	7,2±1,6	2,3±0,8	39,5±8,0	24,2±4,7	0,25±0,03
Preimplantation (the 1st анв 3d days of infusion)								
Killevir 5,0 ml/kg	6	13,3±1,2	9,8±1,4	9,3±1,3	0,5±0,2	28,3±6,0	5,1±1,6	0,29±0,02
Aqueous solution of a DMSO 5,0 ml/kg	7	15,9±1,4	11,7±0,5	10,9±0,8	0,7±0,4	23,9±5,1	6,1±5,0	0,26±0,01
Organogenesis (the 7th and 9th days of infusion)								
Killevir 0,5 ml/kg	5	13,2±0,7	7,0±2,3	6,6±2,1	0,4±0,4	49,4±14,8	5,7±2,7	0,34±0,04
Killevir 1,5 ml/kg	5	13,4±2,1	9,0±2,2	9,0±2,2	0,0±0,0*	32,8±11,1	0,0±0,0*	0,25±0,01
Killevir 5,0 ml/kg	5	16,2±1,3	5,8±1,9	4,4±1,8	1,4±0,80	64,2±14,1	24,1±4,6	0,28±0,02
Aqueous solution of a DMSO 0,5 ml/kg	4	13,3±1,5	10,0±2,1	9,8±2,1	0,3±,03	24,5±13,6	2,5±2,1	0,29±0,02
Aqueous solution of	3	17,3±0,7	6,0±2,1	11,7±1,2	0,3±0,3	30,8±4,6	2,8±1,9	0,27±0,00

3a DMSO 1,5 ml/kg								
Aqueous solution of a DMSO 5,0 ml/kg	3	18,7±4,1	8,0±1,5	5,7±0,3	2,3±1,9	57,1±12,0	29,2±8,9	0,27±0,03
Organogenesis (the 11th and 13th days of infusion)								
Killevir 0,5 ml/kg	4	15,3±2,5	8,5±2,5	8,3±2,5	0,3±0,3	44,3±20,5	3,0±2,1	0,32±0,07
Killevir 1,5 ml/kg	4	14,0±1,1	8,5±2,7	7,0±2,9	1,5±0,5	39,3±20,0	17,7±2,5	0,26±0,01
Killevir 5,0 ml/kg	4	15,3±1,7	8,3±0,9	8,0±0,8	0,3±0,3	45,9±9,4	3,0±1,3	0,27±0,02
Aqueous solution of a DMSO 0,5 ml/kg	3	16,7±3,7	10,0±2,1	1,0±2,1	0,0±0,0*	40,0±11,7	0,0±0,0*	0,27±0,02
Aqueous solution of 3a DMSO 1,5 ml/kg	3	13,0±1,5	6,0±2,1	5,7±2,3	0,3±0,3	53,9±24,5	5,6±2,2	0,29±0,01
Aqueous solution of a DMSO 5,0 ml/kg	4	15,8±1,2	9,0±2,0	7,5±0,9	1,5±0,3	42,9±3,7	16,7±1,8	0,28±0,01
Ossification (the 16th and 18th days of infusion)								
Killevir 5,0 ml/kg4	4	16,0±3,8	8,5±2,6	8,0±2,6	0,3±0,3	46,9±22,7	3,0±3,6	0,22±0,01

Aqueous solution of a DMSO 5,0 ml/kg	4	16,9±1,4	12,5±1,7*	11,3±0,4*	0,7±0,4	12,3±3,5*	8,0±2,6	0,23±0,02
Results of the fetus assessment								
Group	Fetus parameters		Results of the fetus external examination		Results of the fetus osseous system state assessment		Results of the fetus visceral organ state assessment	
	Body mass, g	Cranio caudal size, cm	Quantity of the examined fetuses	Quantity of fetuses with the development anomalies: general / %	Quantity of the examined fetuses	Quantity of fetuses with the development anomalies: general / %	Quantity of the examined fetuses	Quantity of fetuses with the development anomalies: general / %
Control	3,8±0,3	3,8±0,1	72	0/0,0	33	3/9,1	39	0/0
Preimplantation (the 1st анв 3d days of infusion)								
Killevir 5,0 ml/kg	2,6±0,06***	3,3±0,1***	56	0/0,0	27	14/51,9	29	9/31,0
Aqueous solution of a DMSO 5,0 ml/kg	2,6±0,08***	3,2±0,2**	77	1/1,3	39	25/32,5	38	12/15,6
Organogenesis (the 7th and 9th days of infusion)								
Killevir 0,5 ml/kg	2,5±0,06***	3,4±0,05**	32	0/0,0	16	13/81,3	16	6/37,5
Killevir 1,5	2,6±0,08*	3,4±0,09*	45	0/0,0	22	17/77,3	23	6/26,1

ml/kg	**							
Killevir 5,0 ml/kg	2,9±0,1**	3,7±0,2	22	0/0,0	10	7/70,0	12	0/0
Aqueous solution of a DMSO 0,5 ml/kg	2,6±0,2*	3,3±0,3	39	0/0,0	19	14/73,7	20	7/35,0
Aqueous solution of 3a DMSO 1,5 ml/kg	2,8±0,05*	3,4±0,08	35	0/0,0	17	15/88,3	18	9/50,0
Aqueous solution of a DMSO 5,0 ml/kg	2,7±0,1*	3,3±0,2*	17	0/0,0	8	4/50,0	9	3/33,3
Organogenesis (the 11^{th} and 13^{th} days of infusion)								
Killevir 0,5 ml/kg	2,7±0,1**	3,5±0,2	33	1/3,0	15	7/46,7	18	6/33,3
Killevir 1,5 ml/kg	2,9±0,07*	3,6±0,04	28	0/0,0	14	10/71,4	14	2/14,3
Killevir 5,0 ml/kg	2,7±0,08**	3,5±0,06*	32	0/0,0	16	16/100,0	16	6/37,5
Aqueous solution of a DMSO 0,5 ml/kg	2,5±0,06**	3,2±0,1*	30	0/0,0	15	14/93,3	15	2/13,3
Aqueous solution of	2,8±0,2*	3,5±0,2	17	0/0,0	8	7/87,5	9	1/11,1

a DMSO 1,5 ml/kg								
Aqueous solution of a DMSO 5,0 ml/kg	2,7±0,02**	3,5±0,01*	30	0/0,0	15	15/100,0	15	4/26,7
Ossification (the 16th and 18th days of infusion)								
Killevir 5,0 ml/kg	3,8±0,4	4,0±0,2	33	0/0,0	9	0/0	24	0/0
Aqueous solution of a DMSO 5,0 ml/kg	4,1±0,1	4,1±0,05	46	1/2,2	23	1/4,4	23	3/13,1
Note: *p>0,05; **p>0,02; ***p>0,01								

A trustworthy increase of the embryo quantity with a hemorrhage disturbance, characterized by a venous plethora in the liver, especially in a group of animals, receiving the killevir and a OMSO aqueous solution in the 1st and 3d days of pregnancy in a dose of 5,0 ml/kg, was noted at a study of the visceral organ state by a method of the serial sections, practically in all the experimental groups (table 7).

Table 7.

Results of investigation of the fetus visceral organ state after the killevir intravenous infusion

Group	Quantity of fetuses with the visceral organ anomalies (abs. / %)	
	Plethora in the liver vessels	Hemorrhage in the liver
Control	0/0	0/0
Preimplantation (the 1st анв 3d days of infusion)		
Killevir 5,0 ml/kg	7/24,1	2/6,9
Aqueous solution of a DMSO 5,0 ml/kg	11/28,9	1/0,4
Organogenesis (the 7th and 9th days of infusion)		
Killevir 0,5 ml/kg	4/25,0	1/6,3
Killevir 1,5 ml/kg	5/21,7	¼,3
Killevir 5,0 ml/kg	0/0	0/0
Aqueous solution of a DMSO 0,5 ml/kg	4/20,0	3/15,0
Aqueous solution of 3a DMSO 1,5 ml/kg	8/44,4	1/5,6
Aqueous solution of a DMSO 5,0 ml/kg	3/33,3	0/0
Organogenesis (the 11th and 13th days of infusion)		
Killevir 0,5 ml/kg	0/0	6/33,3
Killevir 1,5 ml/kg	1/7,1	1/7,1
Killevir 5,0 ml/kg	5/31,3	0/0
Aqueous solution of a DMSO 0,5 ml/kg	2/13,3	0/0
Aqueous solution of 3a DMSO 1,5 ml/kg	1/11,1	0/0
Aqueous solution of a DMSO 5,0 ml/kg	4/26,7	0/0
Ossification (the 16th and 18th days of infusion)		
Killevir 5,0 ml/kg	0/0	0/0
Aqueous solution of a DMSO 5,0 ml/kg	1/4,3	0/0

An inhibition of the breast bone ossification in all the experimental groups of animals, excluding the embryos, receiving the killevir and a DMSO aqueous solution in the 16th and 18th days of pregnancy, was more frequently revealed at a study of the osseous system development (table 8). There is an apparent contradiction, because the osteogenesis peak was exactly observed in the last days of pregnancy. However, such a contradiction is explained from the view point of an established toxic effect of the killevir and a DMSO injectional form in a period of organogenesis and a general underdevelopment of the fetuses. I.e., in the given case, an inhibition of ossification, especially of the large bones, is exactly connected with it.

Table 8.

Results of investigation of the fetus osseous system development anomalies after the killevir intravenous infusion

Group	Quantity of fetuses with the osseous system anomalies (abs. / %)			
	Inhibition of a breast bone ossification	Inhibition of the ischial bone ossification	Inhibition of a hyoid bone ossification	Inhibition of the metacarpal and metatarsal bone ossification
Control	3/9,1	0/0	0/0	0/0
Preimplantation (the 1st анв 3d days of infusion)				
Killevir 5,0 ml/kg	13/48,2	1/3,7	0/0	0/0
Aqueous solution of a DMSO 5,0 ml/kg	24/61,4	1/2,6	0/0	0/0
Organogenesis (the 7th and 9th days of infusion)				
Killevir 0,5 ml/kg	11/68,8	1/6,3	0/0	1/6,3
Killevir 1,5 ml/kg	17/77,3	0/0	0/0	0/0
Killevir 5,0 ml/kg	6/60,0	1/10,0	0/0	0/0
Aqueous solution of a DMSO 0,5 ml/kg	11/57,9	1/5,3	1/5,3	1/5,3
Aqueous solution of 3a DMSO 1,5 ml/kg	15/88,3	0/0	0/0	0/0
Aqueous solution of a	3/37,5	1/12,5	0/0	0/0

DMSO 5,0 ml/kg				
Organogenesis (the 11th and 13th days of infusion)				
Killevir 0,5 ml/kg	6/40,0	0/0	0/0	1/6,7
Killevir 1,5 ml/kg	9/64,3	0/0	0/0	1/7,2
Killevir 5,0 ml/kg	15/93,8	0/0	0/0	1/6,3
Aqueous solution of a DMSO 0,5 ml/kg	14/93,3	0/0	0/0	0/0
Aqueous solution of 3a DMSO 1,5 ml/kg	5/62,5	0/0	1/12,5	1/12,5
Aqueous solution of a DMSO 5,0 ml/kg	12/80,0	2/13,3	0/0	1/6,7
Ossification (the 16th and 18th days of infusion)				
Killevir 5,0 ml/kg	0/0	0/0	0/0	0/0
Aqueous solution of a DMSO 5,0 ml/kg	1/4,4	0/0	0/0	0/0

Thus, the conducted investigations have permitted to establish a presence of the killevir and a DMSO aqueous solution embryotoxic effect at an intravenous infusion in a preimplantation period, as well as in the different periods of the embryogenesis.

2.3. Results of investigation of the killevir toxic effect on the male gonads

The results of the testis morphofunctional state study are presented in a table 9.

Judging the data, presented in a table, a trustworthy decrease of the testis mass coefficient in a group, rectally receiving the killevir in a dose of 286,0 mg/kg, was revealed at the killevir infusion into the rat males. Besides, a decrease of the prostate mass coefficient is noted in groups, receiving the killevir rectally in doses of 28,6 and 286,0 mg/kg. However, the trustworthy differences in the sperm-

producing epithelium values in all the investigating groups were not revealed at a histologic investigation of the testes.

Table 9.

Morphofunctional values of the white rat testis state after the killevir rectal and intravenous infusion

Value	Control	Intravenous infusion			Rectal infusion		
		Killevir 0,5 ml/kg	Killevir 5,0 ml/kg	Aqueous solution of a DMSO 5,0 ml/kg	Killevir 28,6 mg/kg	Killevir 286,0 mg/kg	Suppository tela of a DMSO 286,0 mg/kg
Mass coefficient of the testes	8,8±0,3	8,7±0,2	9,2±0,3	8,1±0,7	8,0±0,3	7,7±0,2*	8,5±0,4
Mass coefficient of the appendages	4,0±0,6	3,4±0,2	3,4±0,2	3,5±0,2	3,4±0,3	3,0±0,02	3,7±0,5
Mass coefficient of the prostate	3,1±0,4	2,8±0,5	3,0±0,2	2,3±0,6	1,7±0,3*	1,7±0,6	2,5±0,5
Pathologic forms of the spermatozoa, %	37,3±1,9	31,3±1,6	35,1±2,3	34,6±1,2	40,0±1,6	35,7±1,2	42,8±0,04
Index of a spermatogenesis	3,12±0,04	3,08±0,08	3,11±0,04	3,02±0,03	3,09±,003	3,06±0,04	3,03±0,04
Number of small canals with a shelled epithelium, %	0,8±0,6	0,6±0,4	1,6±0,7	2,0±0,4	2,2±0,4	3,0±1,0	1,6±0,5
Average number of the spermatogones,	40,7±1,3	39,4±1,9	40,3±0,9	39,7±0,9	36,0±1,6	39,2±2,2	39,1±1,0

%							
Note: p>0,05							

2.4. Results of investigation of the killevir toxic effect on the female gonads

A daily analysis of the rat vaginal smears during the whole date of observation, during 15 days (3 estral cycles) has demonstrated a correct regular alternation of the estral cycle phases in all the animals, rectally receiving the killevir in a dose of 286 mg/kg of a body mass. No disturbances of the phase alternation and the estral cycle general duration are also revealed in the control animals.

A calculation of the ovary structural elements was made for the more detailed analysis of the female gonad state. Judging the presented in the table data, the killevir preparation infusion into the rat females did not influence the quantitative values of the ovary structural and functional elements.

Table 10.

Results of a quantitative assessment of the rat ovary structural elements after killevir rectal infusion

Structural elements of the ovaries	Control	Killevir 286,0 ml/kg	Suppository tela with a DMSO 286,0 mg/kg
Atretic bodies of the ovary	1186,4±115,7	12458,3±149,6	1088,9±122,8
Graafian vesicles	6,4±0,6	6,8±0,4	6,1±0,6
Folliculi with a layer of the granulous cells	675,2±48,0	744,4±54,8	649,5±64,1
Folliculi with two and more layers of the granulous cells	86,5±8,7	92,3±7,4	82,3±6,2

Thus, the killevir preparation had no toxic effect on the female gonads at a rectal infusion during 3 estral cycles in a dose, corresponding to a 10-fold equitherapeutic one.

CONCLUSION

An experimental study of a reproductive toxicity of the killevir new original preparation, possessing of an antiviral activity and recommended as an antiherpetic preparation, is made.

The killevir substance is a derivative of a fullerene and aminocaproic acid – a fullerenepolyaminocaproic acid. A substance is insoluble in water, that is why a dimethylsulfoxide (DMSO) is used for its dissolution.

The two medicinal forms of the killevir: the suppositoria and a concentrate for injections are proposed for use.

The experiments are conducted on the outbreeding white rats, acquired in the Central nursery of the laboratory animals of the RAMS, department “Kryukovo”. A study of the killevir embryotoxic and teratogenic effect was made in the

antenatal period. A study of the killevir gonadotoxic effect has been made on the males during 30 days and on the females – during 15 days.

A suppository mass of the killevir has been rectally infused into the rat females in doses of 28,6 (equitherapeutic), 85,8 and 286,0 mg/kg of a body mass from the first to twentieth days of pregnancy. The killevir was intravenously infused into the pregnant females in doses of 0,5 (equitherapeutic); 1,5 and 5,0 ml/kg according to the following scheme:

for a study of an influence on a preimplantation period: in the 1^{st} and 3^{d} days of pregnancy;

for a study of an influence on the early period of organogenesis: in the $7^{th}$and 9^{th} days of pregnancy;

for a study of an influence on the late period of organogenesis: in the 11^{th} and 13^{th} days of pregnancy;

for a study of an influence on the osseous system development: in the 16^{th} and 18^{th} days of pregnancy.

A suppository tela with a DMSO or a DMSO aqueous solution in a quantity and concentrations, corresponding to its content in the corresponding medicinal forms, were infused into the separate groups of animals at all the stages of investigation. Besides, a water for injections (control) was infused into supplementary groups of animals. The female behavior, clinical signs and dynamics of a body mass have been registered during the preparation infusion. At the 20^{th} day of pregnancy the animals were killed and the fetus state analysis was made.

The killevir in doses of 28,6 and 286,0 mg/kg and a suppository tela with a DMSO in a dose of 286,0 mg/kg have been daily infused during 30 days rectally and intravenously in doses of 0,5 and 5,0 ml/kg and a DMSO aqueous solution in a dose of 5,0 ml/kg once at the first day of experiment for a study of a toxic effect on the male gonads. A water for injections was infused into the control animals. The animals were daily examined and weekly weighed, after that they were killed and an autopsy was made.

The killevir has been daily infused during 15 days rectally and in a dose of 286,0 mg/kg for a study of a toxic effect on the female gonads. A suppository tela with a DMSO in a dose of 286,0 mg/kg was infused into the control.

An assessment of the rat generative function state has revealed a trustworthy increase of the implantation site and living fetus quantity in a group of animals, receiving the preparation in a dose of 85,8 mg/kg of a body mass, at a rectal infusion of the killevir. An alternation of the given values is not a dose-dependent. An analysis of the other values has not revealed the differences between the experimental and control animals.

An analysis of the fetus visceral organ state by the serial section method has demonstrated a trustworthy increase of the fetus quantity with liver vessel plethora in groups, where the rats were rectally receiving the killevir in doses of 85,8 mg/kg and 286,0 mg/kg. Upon the whole, an increased level of the hemorrhage appearance was observed in these groups. No substantial differences with the control are noted on the other anomalies.

A trustworthy inhibition in the breast bone ossification is revealed at a study of the osseous system development in a group of embryos, the mothers of which were rectally receiving the killevir in a dose of 286,0 mg/kg. Besides, the higher value of the separate bone ossification inhibition is noted at an effect of a suppository tela with a DMSO.

It was established at an assessment of the intravenous infusion results, that such parameters of a generative function assessment, as a quantity of the yellow bodies in the ovaries and a quantity of the implantation sites, and, consequently, a preimplantation mortality of the fetuses, were not statistically different. An exclusion was a group, intravenously receiving a DMSO aqueous solution in the 16^{th} and 18^{th} days of pregnancy in a dose of 5,0 ml/kg. A trustworthy increase of the implantation site and living fetus quantity is noted in the given group animals and a preimplantation mortality is trustworthy decreased. There were no statistically meaningful differences for all the other experimental groups.

A trustworthy decrease of the embryo body mass and craniocaudal size in all the experimental groups, regardless of a dose and date of the killevir or a DMSO solution intravenous infusion, was noted, excluding the groups, into which the killevir and a DMSO aqueous solution were infused in the 16^{th} and 18^{th} days of pregnancy. It testifies to the embryo- and fetotoxic effect, i.e. an influence in a period of organogenesis. Besides, a trustworthy increase of the embruo quantity with a hemorrhage disturbance, characterized by a venous plethora in the liver, especially in a group of animals, receiving the killevir and a DMSO aqueous solution in the 1^{st} and 3^{d} days of pregnancy in a dose of 5,0 ml/kg, was noted at a section method, practically in all the experimental groups.

An inhibition of the breast ossification in all the experimental groups of animals, excluding the embryos, receiving the killevir and a DMSO aqueous solution in the 16^{th} and 18^{th} days of pregnancy, was more frequently revealed at a study of the osseous system development. There is an apparent contradiction, because the osteogenesis peak is observed exactly in the last days of pregnancy. However, such a contradiction is explained from the view point of the established toxic effect of the killevir and a DMSO injectional form in a period of organogenesis and a general underdevelopment of the fetuses. I.e., an inhibition of ossification, especially of the large bones, is connected with it in the given case.

A trustworthy decrease of the testis mass coefficient in a group, rectally receiving the killevir in a dose of 286,0 mg/kg, was revealed at a study of the killevir toxic effect on the rat male gonads. Besides, a decrease of the prostate mass coefficient is noted in the groups, rectally receiving the killevir in doses of 28,6 and 286,0 mg/kg. However, no trustworthy differences in the sperm-producing epithelium values were not revealed at the testis histologic investigation in all the investigating groups.

A daily analysis of the rat vaginal smears during the whole date of observation, during 515 days (3 estral cycles) has demonstrated a correct regular alternation of the estral cycle phases in all the animals, rectally receiving the killevir in a dose of 286,0 mg/kg of a body mass. No disturbances of the phase

alternation and the estral cycle general duration are also revealed in the control animals.

Besides, the killevir preparation infusion into the rat females did not influence the quantitative values of the ovary structural and functional elements.

Thus, the meaningful signs of a damaging effect are not established at an experimental study of a reproductive toxicity of the killevir preparation in a form of suppositoria for a rectal infusion. However, the noted increase of the liver plethora at a use of the 3 and 10-fold equitherapeutic doses deserves an attention.

A disturbance of a fetus general development, expressed in the mass and size decrease, including an effect of the preparation equitherapeutic dose, is established at a study of the killevir injectional form.

There are all grounds to presume, that the revealed embryotoxic effects are caused by a DMSO. At the same time, the preparation use is connected with a risk for a fetus, considering the fact, that a DMSO is an integral part (a substance solvent) of the killevir preparation medicinal forms.