FEDERAL MEDICOBIOLOGIC AGENCY

**FEDERAL STATE INSTITUTION OF SCIENCE
SCIENTIFIC RESEARCH CENTRE OF TOXICOLOGY AND
HYGIENIC REGLAMENTATION OF THE BIOPREPARATIONS
(FSIS, SRC of TBP, FMBA of Russia)**

APPROVED
by Deputy director
of the SRC of TBP
on a scientific work,
M.D., professor
_____ N.R. Dyadishev
2005.

Seal: FSIS, SRC of TBP, FMBA of Russia

REPORT
Study of the Killevir preparation substance and medicinal form mutagenous effect
(Agreement CT 14/2005)

M.D.
Head of investigations A.A. Denisov

Serpukhov - 2005

LIST OF EXECUTORS

M.D., Head of the molecular genetics and immunology department	A.A. Denisov
Candidate of biological sciences Leading scientific worker	L.V. Mikhova
Candidate of biological sciences Leading scientific worker	A.I. Marchenko
Senior scientific worker	O.A. Karpova
Scientific worker	A.V. Tretiyakova
Junior scientific worker	S.V. Melnickova
Junior scientific worker	O.M. Rybakova
Junior scientific worker	S.A. Popova
Laboratory assistant-investigator	E.N. Sokolova
Laboratory assistant-investigator	I.V. Kozlova
Laboratory assistant-investigator	L.A. Intse

LIST OF PROVISIONAL ABBREVIATIONS

DDDTDP – 2,7-diamino- 4,9- dioxy, 5,10- dioxo- 4,5,9,10 tetra-hydro- 4,9-diasopyrene

DMSO – dimethylsulfoxide

IMAM – incomplete microsomal activating mixture

NMU – nitrosamethylurea

CMAM – complete microsomal activating mixture

PF – proflavine

CT - cytoxan

PAPER

Volume of a report – 22 pages, 7 tables, references – 7 names

Key words: fullerenepolyaminocaproic acid, medicinal preparation Killevir, mutagenous effect, chromosomal aberrations, strain Salmonella typhimurium, the Ames' test

The aim of the present work is a detection and quantitative assessment of the killevir preparation substance and two medicinal form potential cytogenetic activity: injectional one (for the intravenous injections) and in a form of suppositoria (rectal).

A substance of the killevir preparation is a derivative of a fullerene and aminocaproic acid: a fullerenepolyaminocaproic acid. A substance is weakly soluble in water and well soluble in a DMSO, that's why the both medicinal forms contain a DMSO.

A method of the chromosomal aberration consideration in the thermal-blood animal bone marrow cells and the Aimes' test on the indicator strains of Salmonellas were used for a mutagenous effect assessment.

A consideration of the chromosomal aberrations was made in the mouse male and female bone marrow cells of the $C_{57}B1/6$ line after the killevir preparation medicinal form effect in two doses: a proximate to the recommended for the clinical tests therapeutic dose (according to a substance content – 0,7 mg/kg for an injectional form and 0,14 mg/kg for the suppositoria) and 10-times its exceeding. A dioxidine, which is a mutagen of a prooxidant effect type in a dose of 300 mg/kg at a single intraperitonealy infusion, was used as a positive control. The negative control groups are presented by the animals, receiving a solvent, corresponding to each medicinal form: a DMSO 3% or a DMSO in a vitepsol.

It was established, that a single intraperitonealy infusion of the killevir preparation injectional form in a therapeutic dose did not cause a statistically meaningful increase of the aberrant cell share in the bone marrow (2,20±0,58% - an experiment, 1,20±0,37% - a negative control). The killevir preparation injectional form in a dose, 10-times exceeding the therapeutic one, was causing a trustworthy more than 3-times increase of the cell shape with the structural disturbances (4,20±0,58% - an experiment, 1,20±0,37% - a negative control) at the same experiment. A five-fold rectal infusion of the killevir preparation (in a form of suppositoria) in a therapeutic dose did not cause an increase of the affected cell shape in the experimental mouse bone marrow (females: 1,20±0,49% - an experiment, 1,00±0,45% - a negative control; males: 1,00±0,32% - an experiment, 0,80±0,20% - a negative control).

Thus, it is established, that the killevir preparation on a basis of a fullerenepolyaminocaproic acid (injectional form) in high doses (according to a substance content – 7,0 mg/kg, a dose, 10-times exceeding the therapeutic one) possess of an ability to induce the mutations in the thermal-blood animal somatic cells. The killevir preparation medicinal forms (injectional and suppositoria rectal)

do not possess of mutagenous effect on the thermal-blood animal somatic cells in doses, proximate to the recommended for the clinical tests ones (according to a substance content – 0,7 mg/kg for an injectional form and 0,14 mg/kg for the suppositoria).

In the Ames' test, a mutagenous effect of the killevir substance and preparation (injectional form) was studied on the standard test-strains Salmonella typhimurium TA98, TA100, TA1537, TA1950 and TA1534. It was established, that the killevir preparation substance (a fullerenepolyaminocaproic acid) and injectional form do not possess of a mutagenous activity.

CONTENTS

INTRODUCTION

The killevir medicinal preparation substance (a fullerenepolyaminocaproic acid) and two medicinal forms: suppositoria rectal and a concentrate for injections are presented for investigation.

The results of the killevir preparation substance and medicinal form mutagenous activity investigation are presented in the present report it two tests:

- by a method of the chromosomal aberration consideration in the thermal-blood animal bone marrow cells,
- in the Aimes' test on the indicator strains of Salmonellas.

The work is accomplished according to the "Methodical recommendations of the RF MPH Pharmacologic committee "Assessment of the pharmacologic preparation mutagenous properties" [1].

1. MATERIALS AND METHODS

Laboratory animals

The experiments are performed on the inbreeding mice of the C_{57}BL/6 line, males and females at the age of 2 months. A maintenance of the animals in the TBP SRC vivarium corresponded to the sanitary rules, approved by the USSR MPH on 73.07.06, on arrangement equipping and maintenance of the experimentally-biological clinic (vivaria) [7].

The rodents were fed with a full-rational mixed feed, a plumbing water was used for drinking. The animals have passed a quarantine and acclimatization in the conditions of vivarium during not less than 14 days.

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

Preparation of the medicinal form solutions for infusion into animals

Presented for investigation:

1) the killevir preparation substance (a fullerenepolyaminocaproic acid);
2) a concentrate of the killevir medicinal preparation (injectional form, to dissolve in 30 ml of the water for injections before infusion, the received solution is a Solution A) of the following composition:

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

3) the killevir medicinal preparation (suppositoria rectal) of the following composition:

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

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

Medicinal preparation killevir (injectional form)

The presumed for the tests in a clinic therapeutic dose of the killevir preparation (injectional form, according to a substance content) is 0,7 mg/kg (1 TD), a dose, 10-times its exceeding, is 7 mg/kg (10 TD).

10 TD: At a mouse body mass of 20 g, it is 0,14 mg/specimen (according to a substance content). 0,14 mg of a substance is contained in 0,08 ml of the Solution A. The pointed volume of the Solution A was brought to 0,6 ml with a DMSO solution 3% on a water for injections (a dose/specimen).

1 TD: At a mouse body mass of 20 g, it is 0,014 mg/specimen (according to a substance content). After the Solution A 10-fold dilution (the received solution is a Solution B), 0,014 mg of a substance is contained in 0,08 ml of the Solution B. The pointed volume of the Solution И was brought to 0,6 ml with a DMSO solution 3% on a water for injections (a dose/specimen).

Medicinal preparation killevir (suppositoria rectal)

The presumed for the tests in a clinic therapeutic dose of the killevir preparation (suppositoria rectal, according to a substance content) is 0,14 mg/kg. At a mouse body mass of 20 g, it is 0,0028 mg/specimen (according to a substance content), the pointed dose in contained in 0,56 mkl of a medicinal form. A medicinal form with a use of placebo for a rectal form was 10-times diluted (Dilution C), infused by 6 mkl of the Dilution C to a specimen.

Preparation of the bone marrow preparations

A 0,025% colchicines solution ("Fluka") in a volume of 0,3 ml (0,075 mg/mouse, 3,75 mg/kg) was singly intraperitonealy infused into mice 2,5 hours before killing for a metaphasic material accumulation. In 2,5 h the animals were killed in a carbonate gas atmosphere and they started to prepare the chromosomal preparations. The chromosomal preparations for a cytogenetic analysis were received according to the generally-accepted methods [5]. The bone marrow cells have been washed off the femoral bones with a warm physiologic salt of a potassium chloride (0,56% solution) during 10 min., fixed with an alcohol-acetic mixture (3:1) and the received cellular suspensions were applied to the humid cooled subject glasses. The preparations have been fixed in a burner flame, stained with a methylrosanilinum chloride solution 0,5% during 10 min. The received preparations (by two glasses from each animal) were ciphered and scrutinized under a luminous microscope immersional system.

The Ames' test conduction

The indicator strains Salmonella typhimurium TA1534, TA1537, TA1950, TA98, TA100 from the microorganism collection of the Serpukhov city TBP SRC have been used during the tests.

The strains were stored in ampules in a lyophilically dried state as well as in a layer of a plain agar 0,6% under a sterile Vaseline oil. The cultures have been inoculated to a mowed plain agar 2% before the experiment beginning. The cultures were inoculated to a beef-extract broth from the jambs. 25 mkg/ml of an ampicillin were added to a plain agar or a broth for the strains TA100 and TA98. An accordance of the strains to a genotype was periodically checked at inoculations to the new jambs.

Characteristics of the Salmonella typhimurium strains		
Strain	Type of mutation	R-factor
TA1534	Shift of a checking frame	—
TA1537	Shift of a checking frame	—
TA1950	Shift of the base pairs	—
TA98	Shift of a checking frame	+
TA100	Substitution of bases, shift of a checking frame	+

The used nutrient media:

- a plain agar 0,6% and 2,0%; the agar-agar aqueous solution 2%.
- a minimum agar 1,5%: an aqueous agar – 300 ml, a minimum liquid medium – 100 ml, a glucose solution 20% - 10 ml, a solution $MgSO_4 \times 7H_2O$ – 2 ml.
- a semiliquid minimum agar 0,7%: an agar – 7 g, NaCl – 6 g, a distilled water is up to 1 l.
- an upper semiliquid semidressed agar: a semiliquid minimum agar (80 ml), 10 ml of a solution L-histidine 0,5 mM, 10 ml of a biotin solution 0,25 mM. An assessment of the killevir substance or preparation aqueous solution dilution toxicity, relating to the Salmonella test-strains, was made for consideration of a solvent influence on the further experiment results. For that purpose, 0,1 ml of the bacterium suspension of 2×10^9 m.kl/ml and 0,1 ml of the killevir substance or preparation aqueous solutions in different concentrations (0,1 ml of a distilled water was added to the control tests instead of them) were brought into the test-tubes with 2 ml of a melted semidressed agar 0,7%. They have been exponated for 40 min at 37°C and poured to a layer of a thickened plain agar. In 48 hours of incubation, a consideration of the S. typhimurium strain cultivated colonies at 37°C was made . The cell viability was expressed in percents.

A postmitochondrial supernatant of the rat liver homogenate (fraction S9), containing the microsomes, has been receiving during 3 days after a Phenobarbital intraperitonealy infusion into the animals by 0,5 ml, counting 80 mg/kg.

6 mg of the S-9 fraction protein, the NADPh 4 mM, a glucose – 6 – phosphate 5 mM, the KCl 33 mM, the $MgCl_2$ 8 mM and 0,2 M of the pH 7.4 phosphatic buffer were contained in 1 ml of a complete microsomal activating mixture (CMAM).

The corresponding quantities of the cofactor solvent were added in case of the incomplete microsomal activating mixture (IMAM) formation instead of a cofactor (NADF, glucoso – 6 phosphate).

0,1 ml of the killevir substance or preparation in the necessary concentration, then 0,1 ml of the bacterium suspension were brought into the test-tubes with a selective semidressed agar (0,7%) for a mutagenous activity study. After that, 0,5 ml of a microsomal activating mixture was brought into. The test-tube contents was rapidly mixed and poured to a layer of the lower minimum agar in the Petri

dish. A duration of a semiliquid agar pouring in the dishes did not exceed 10-15 sec. The dishes were left at a room temperature for 30-40 min. and agar complete thickening. A consideration of results was made in 48-72 hours of incubation.

In the control variant, the CMAM and IMAM were brought into the upper semiliquid agar layer together with the bacterium suspension, into the corresponding volume with a sterile distilled water. The substances, including the mutations in the corresponding test-strains at a presence or lack of the activation conditions, were used as the positive controls. The 2,7 – diamino-4,9-dioxy, 5,10-dioxo-4,5,9,10 tetra-hydro- 4,9 – diasopyrene (DDDTDP), 100 mkg/dish for the strains TA1534 and TA98, a nitrosamethylurea (NMU), 100 mkg/dish for the strains TA1950 and TA100, a proflavine (PF), 500 mkg/dish for a strain TA1537 were used for the IMAM variants. An activity of a fraction S-9 was controlled, parallely using in the variants with the CMAM and IMAM a cytoxan (CT), 500 mkg/dish.

By three dishes were used in each control and experimental variants. The results were considered at a presence of a mutagenous effect in all the variants of a positive control. The experiment was three-times repeated.

Statistical treatment

The Student's criterium was used at a statistical treatment of the received experimental data [2].

2. RESULTS OF INVESTIGATIONS

A cytogenetic activity of the killevir preparation (injectional form) was studied at a single intraperitonealy infusion, the killevir preparation (suppositoria rectal) – at a five-fold rectal infusion.

Experimental groups of animals

Stage	№ of a group	Conditions of experiment			Mice $C_{57}BL/6$, quantity	
		Infusion	Infused substance	Dose	Males	Females
I	1.1	Intraperitonealy, singly	Killevir (injectional form)	7 mg/kg	5	—
	1.2			0,7 mg/kg	5	—
	1.3		DMSO 3% (negative control)	—	5	—
	1.4		Dioxidine (positive control)	300 mg/kg	5	—
II	2.1	Rectally five-fold	Killevir (suppositoria rectal)	0,14 mg/kg	5	5
	2.2					
	2.3-2.4		Placebo (negative	—	5	5

			control)			
Totally:					30	30

A selection of the preparation testing doses was based on the elaborator data of the presumed in the clinical tests therapeutic doses: according to a substance content – 0,7 mg/kg for an injectional form and 0,14 mg/kg – for the suppositoria rectal. At the first stage of experiment, the preparation injectional form in a dose, corresponding to a daily therapeutic one for a human and in a dose, 10-times its exceeding, was singly intraperitonealy infused to the males only. At the second stage, the testing preparation in a form of suppositoria has been rectally daily infused into the males and females during 5 days in a dose, corresponding to a daily therapeutic one for a human. A dioxidine, which is a mutagen of a prooxidant type of effect, was used as a positive control in a dose of 300 mg/kg at a single intraperitonealy infusion. The groups of a negative control are presented by the animals, receiving a solvent, corresponding to each medicinal form: a DMSO 3% or a DMSO in a vitepsol. By 5 animals were taken to each experimental group.

At examination of the mouse bone marrow received cytologic preparations, each metaphasic plate was analyzed to a presence of the chromosomal aberrations (fragments and exchanges), selecting the round metaphasic plates without the chromosome application with a modal number of 40. The achromatic gaps were not considered. Not less than 100 metaphasic plates from each animal were analyzed at examination of the bone marrow preparations. A number of the single and pair fragments, the chromatid and chromosome exchanges, a number of cells with the multiple damages was considered. A share of the damaged cells for each animal and in a group as a whole was calculated. A statistical treatment was made according to the generally-accepted recommendations [2].

2.1. STUDY OF THE KILLEVIR PREPARATION MUTAGENOUS EFFECT (INJECTIONAL FORM) AT A SINGLE INTRAPERITONEAL INFUSION

The results of the mouse bone marrow conducted cytogenetic investigation are summarized in a Table 1.

No statistically meaningful increase of a share of cells with the chromosome structural disturbances is noted at a single intraperitonealy effect of the preparation daily therapeutic dose (0,7 mg/kg), compared to the control group (2,20±0,58% and 1,20±0,37%, respectively). The preparation infusion in a dose, a cut above the presumed therapeutic one (7 mg/kg) has caused a more than three-fold trustworthy increase of the damaged cell share (4,20±0,58%). A character of the chromosome damages in the control and experimental groups did not differ: the basic mass of all the reconstructions were the chromatid type aberrations (the terminal deletions, Fig. 1). A share of the damaged cells was 23,40±4,00% in a positive control group after the dioxidine effect (300 mg/kg), possessing of a mutagenous effect (300 mg/kg), possessing of a mutagenous activity, the main types of damages were the multiple chromosomal aberrations.

Fig. 1. The main types of the chromosome structural disturbances, encountering in the mouse bone marrow cells after the preparation infusion on a basis of a fullerenepolyaminocaproic acid.

Table 1.

Data of the mouse bone marrow cell genetic screening after the killevir preparation injectional medicinal form infusion

Preparation, dose	№ of a mouse	Quantity of investigated cells	Quantity of cells with aberrations	Quantity of aberrations			Cells mul aberr
				Single fragments	Pair fragments	Exchanges	
Killevir, 7 mg/kg	1	100	5	5	1	0	
	2	100	6	6	0	0	
	3	100	3	3	0	0	
	4	100	4	2	2	0	
	5	100	3	3	0	0	
		500	21	19	3	0	
Killevir, 7 mg/kg	6	100	1	1	0	0	
	7	100	3	2	1	0	
	8	100	2	2	0	0	
	9	100	1	0	1	0	
	10	100	4	4	0	0	
		500	11	9	2	0	
Negative control (DMSO, 3%)	11	100	1	1	0	0	
	12	100	0	0	0	0	
	13	100	2	1	1	0	
	14	100	1	1	0	0	
	15	100	2	2	0	0	
		500	6	5	1	0	
Positive	16	100	22	7	0	0	1

[1] statistically trustworthy difference with a negative control

control (dioxidine, 300 mg/kg)	17	100	24	5	1	0	1
	18	100	29	3	1	0	2
	19	100	18	5	0	0	1
	20	100	24	6	2	0	1
		500	117	26	4	0	8

2.2. STUDY OF THE KILLEVIR PREPARATION MUTAGENOUS EFFECT (SUPPOSITORIA RECTAL) AT A FIVE-FOLD RECTAL INFUSION

A five-fold rectal infusion of the killevir preparation (suppositoria rectal) in a daily therapeutic dose (0,14 mg/kg) did not cause a statistically trustworthy increase of the damaged cell quantity compared to the group of animals, receiving a placebo (Table 2). A quantity of cells with the chromosomal aberrations in the mouse bone marrow in experiment was: in females – 1,20±0,49% and in males – 1,00±0,45%, which did not differ from the control level (1,00±0,45% and 0,80±0,20%, respectively).

Table 2.

Data of the mouse bone marrow cell genetic screening after a five-fold infusion of the killevir preparation (suppositoria rectal).

Sex	Preparation, dose	№ of a mouse	Quantity of investigated cells	Quantity of cells with aberrations	Quantity of aberrations		
					Single fragments	Pair fragments	Exchanges
♀	Killevir, 0,14 mg/kg	26	100	1	2	0	0
		27	100	0	0	0	0
		28	100	3	3	0	0
		29	100	1	1	0	0
		30	100	1	0	1	0
			500	6	6	1	0
	Negative control (placebo)	36	100	2	1	1	0
		37	100	0	0	0	0
		38	100	0	0	0	0
		39	100	1	1	0	0
		40	100	2	2	0	0
			500	4	4	1	0
♂	Killevir, 0,14 mg/kg	21	100	1	1	0	0
		22	100	2	2	0	0
		23	100	0	0	0	0
		24	100	1	1	0	0
		25	100	1	1	0	0
			500	5	5	0	0
	Negative control	31	100	1	1	0	0
		32	100	1	0	1	0

	(placebo)	33	100	1	1	0	0
		34	100	1	1	0	0
		35	100	0	0	0	0
			500	4	3	1	0

The chromosomal reconstructions, revealed in the bone marrow cells at the killevir preparation multiple effect, did not differ from the chromosomal reconstruction type, encountered in mice after a placebo infusion and are mainly presented by the chromatid type aberrations (terminal deletions, Fig. 1).

Thus, no increase of the chromosome damage quantity and the multiple aberration appearance in the both sex experimental mouse bone marrow cells is established at the killevir preparation five-fold infusion (suppositoria rectal) is a daily therapeutic dose (0,14 mg/kg). A maximum share of the damaged cells in the separate specimen bone marrow was 3% (i.e. <5%). In such cases, the repeated or extended tests for the proposed therapeutic doses of the preparation with a use of the given test are not necessary and a lack of a mutagenous activity is considered to be established. The killevir preparation (suppositoria rectal) on a basis of a fullerenepolyaminocaproic acid does not possess of a mutagenous activity relating to the thermal-blood animal cells.

2.3. STUDY OF THE KILLEVIR PREPARATION SUBSTANCE MUTAGENOUS ACTIVITY (A FULLERENEPOLYAMINOCAPROIC ACID) IN THE AMES' TEST

An assessment of the substance aqueous solution dilution toxicity was made at the first stage of investigations relating to the Salmonella test-strains for consideration of a solvent influence on the subsequent experiment results.

Table 3.

Viability of the S. typhimurium strains (%) in the fullerenepolyaminocaproic acid aqueous solutions, containing a DMSO 3%

Quantity of a testing substance, mkg/dish	STRAIN				
	TA98	TA100	TA1537	TA1950	TA1534
0	100	100	100	100	100
0,1	100	100	100	100	100
1	100	100	100	100	100
10	100	100	100	100	100
100	100	100	100	100	100
165	100	100	100	100	100

The results of a study of the fullerenepolyaminocaproic acid aqueous solution cytotoxic effect on the Salmonella cells have demonstrated, that a DMSO concentration increase in the acid solution up to 4% is leading to a cytotoxicity

appearance (Tables 3, 4). A survival rate of the bacterial strains at a DMSO concentration 3% in solutions did not differ from the control and was 100% (Table 3). Thus, judging from a fullerenepolyaminocaproic acid concentrate, presented for the tests, the maximally achievable dose of the active substance in a solution at the Ames' test organization, was a dose of 165 mkg/dish.

Table 4.

Viability of the S. typhimurium strains (%) in the fullerenepolyaminocaproic acid aqueous solutions, containing a DMSO 4%

Quantity of a testing substance, mkg/dish	STRAIN				
	TA98	TA100	TA1537	TA1950	TA1534
0	72	68	71	72	64
0,1	68	62	72	68	56
1	61	59	68	67	58
10	59	62	63	65	71
100	69	64	65	68	60
165	71	68	61	63	63

At the second stage, a genotoxic effect of a substance on the Salmonella indicator strains was assessed in the Ames' test. It was demonstrated, that a fullerenepolyaminocaproic acid in doses, not exceeding 165 mg/dish, does not induce the mutations in the S. typhimurium strains (Table 6). The positive controls were effectively inducing the mutations in the corresponding Salmonella strains in conditions of the incomplete and complete metabolic activation. All the positive controls (quality mutagens) have demonstrated a distinctly expressed mutagenous effect, which testifies to a good sensitivity of the test-strains to the mutagen effect.

2.4. STUDY OF THE KILLEVIR PREPARATION MUTAGENOUS ACTIVITY (INJECTIONAL FORM) IN THE AMES' TEST

At the first stage of investigations, an assessment of the killevir preparation aqueous solution dilution toxicity relating to the Salmonella test-strains for consideration of a solvent influence on the subsequent experiment results was made. The results of a study of the killevir preparation aqueous solution cytotoxic effect on the salmonella cells have demonstrated, that the solutions do not possess of a toxic effect in the investigating ranges of concentrations (Table 5). A survival rate of the bacterial strains did not differ from the control and was 100%.

At the second stage, a genotoxic effect of the killevir preparation on the Salmonella indicator strains was assessed in the Ames' test. Judging the data, presented in a table 7, the mutations in the S. typhimurium strains do not induce in the tested doses. The positive controls were effectively inducing the mutations in the corresponding Salmonella strains in conditions of the incomplete and complete metabolic activation. All the positive controls (quality mutagens) have

demonstrated a distinctly expressed mutagenous effect, which testifies to a good sensitivity of the test-strains to the mutagen effect.

Table 5.

Viability of the S. typhimurium strains (%) in the killevir preparation aqueous solutions (injectional form), containing a DMSO 3%

Quantity of a testing substance, mkg/dish	STRAIN				
	TA98	TA100	TA1537	TA1950	TA1534
0	100	100	100	100	100
0,1	100	100	100	100	100
1	100	100	100	100	100
10	100	100	100	100	100
100	100	100	100	100	100
165	100	100	100	100	100

Thus, the killevir preparation (injectional form), as well as the preparation substance (a fullerenepolyaminocaproic acid), does not possess of a mutagenous effect, according to the Ames' test data, on the indicator strains of the Salmonella typhimurium bacteria.

Table 6.
Investigation data of the substance mutagenous activity (a fullerenepolyaminocaproic acid) in the aqueous solutions, containing a DMSO 3%, in the Ames' test.

Investigating substance	Dose, mkg/dish	Quantity of colonies/dish[2]									
		Strain TA100		Strain TA1534		Strain TA1537		Strain TA1950		Strain TA98	
		IMAM	CMAM	IMAM	CMAM	IMAM	CMAM	IMAM	CMAM	IMAM	CMAM
Substance	165	54±6	49±6	23±3	36±3	22±4	37±4	20±3	26±3	21±3	31±4
	100	57±6	52±5	19±3	31±3	26±3	35±3	21±2	28±4	23±4	33±3
	10	49±5	54±5	21±2	32±3	24±3	36±4	19±3	26±3	24±2	30±4
	1	45±7	55±6	20±2	37±4	23±4	37±4	21±2	27±3	21±3	32±3
	0,1	50±7	55±6	21±3	32±3	25±3	36±3	22±3	28±3	24±5	31±3
	0	46±6	56±6	22±3	35±4	24±4	39±4	20±2	24±5	19±3	28±4
NMM[3]	100	1845±69	—[4]	—	—	—	—	1243±58	—	—	—
DDDTDP	100	—	—	332±24	—	—	—	—	—	320±39	—
Proflavine	500	—	—	—	—	51±7	—	—	—	—	—
Cytoxan	500	44±6	352±34	21±5	452±41	76±6	268±24	42±5	268±33	62±8	264±29

[2] Note: a confidential interval is calculated with a 95% probability
[3] Note: abbreviations: NMM – a nitrosamethylurea, DDDTDP – 2,7-diamino-4,9-dioxy,5,10-dioxo-4,5,9,1- tetra-hydro-4,9-diasopyrene, IMAM – incomplete microsomal activating mixture, CMAM – complete microsomal activating mixture.
[4] Note: "—" – the given variant in investigation was absent.

Table 7.
Data of the killevir preparation mutagenous activity investigation (injectional form) in the Ames' test

Investigating substance	Dose, mkg/dish	Quantity of colonies/dish[5]									
		Strain TA100		Strain TA1534		Strain TA1537		Strain TA1950		Strain TA98	
		IMAM	CMAM	IMAM	CMAM	IMAM	CMAM	IMAM	CMAM	IMAM	CMAM
Killevir	1000	53±5	54±6	21±4	33±3	24±4	38±4	19±3	28±3	22±5	34±3
	100	56±6	56±6	20±3	32±3	27±3	37±4	17±2	29±4	24±4	32±2
	10	49±5	55±5	20±3	30±3	25±3	37±5	17±3	27±3	21±2	31±4
	1	46±5	55±6	21±4	31±4	24±4	38±2	18±2	28±4	19±3	29±3
	0,1	48±6	55±6	20±3	33±3	27±3	37±3	17±3	29±2	23±5	33±3
	0	45±5	56±6	21±3	32±4	27±4	37±3	17±2	28±3	20±3	30±4
NMM[6]	100	1824±76	—[7]	—	—	—	—	1326±35	—	—	—
DDDTDP	100	—	—	365±29	—	—	—	—	—	370±21	—
Proflavine	500	—	—	—	—	55±6	—	—	—	—	—
Cytoxan	500	46±6	352±34	23±5	484±37	60±4	256±21	38±6	258±31	58±7	286±27

[5] Note: a confidential interval is calculated with a 95% probability
[6] Note: abbreviations: NMM – a nitrosamethylurea, DDDTDP – 2,7-diamino-4,9-dioxy,5,10-dioxo-4,5,9,1- tetra-hydro-4,9-diasopyrene, IMAM – incomplete microsomal activating mixture, CMAM – complete microsomal activating mixture.
[7] Note: "—" – the given variant in investigation was absent.

CONCLUSION

A mutagenous activity of the killevir preparation substance and two medicinal forms; injectional (for the intravenous injections) and in a form of suppositoria (rectal) was studied. The killevir preparation substance is a fullerenepolyaminocaproic acid (a derivative of a fullerene and aminocaproic acid).

A method of the chromosomal aberration consideration in the thermal-blood animal bone marrow cells and the Ames' test on the Salmonella indicator strains were used for a mutagenous effect assessment.

A consideration of the chromosomal aberrations was made in the bone marrow cells of the C57B1/6 line mouse males and females after the killevir preparation medicinal form effect in two doses: a proximate to the recommended for the clinical tests therapeutic dose (0,7 mg/kg for an injectional form and 0,14 mg/kg for suppositoria) and in a dose, 10-times its exceeding.

It was established, that a single intraperitonealy infusion of the preparation injectional form in a therapeutic dose did not cause the statistically meaningful increase of the aberrant cell share in the bone marrow (2,20±0,58% - an experiment, 1,20±0,37% - a negative control). At the same experiment, the killevir preparation injectional form in a dose, 10-times exceeding the therapeutic one, was causing a trustworthy more than 3-times increase of the cell share with the chromosome structural disturbances (4,20±0,58% - an experiment, 1,20±0,37% - a negative control). A five-fold rectal infusion of the killevir preparation (suppositoria) in a therapeutic dose did not cause an increase of the damaged cell share in the experimental mouse bone marrow (females: 1,20±0,49% - an experiment, 1,00±0,45% - a negative control; males: 1,00±0,32% - an experiment, 0,80±0,20% - a negative control).

Thus, its established, that the killevir preparation (injectional form) in high doses (7,0 mg/kg – a dose, 10-times exceeding the therapeutic one) possesses of an ability to induce the mutations in the thermal-blood animal somatic cells. The killevir preparation medicinal forms (injectional and suppositoria rectal) do not possess of a mutagenous effect on the thermal-blood animal somatic cells in doses, proximate to the recommended for the clinical tests ()0,7 mg/kg – for an injectional form and 0,14 mg/kg – for suppositoria).

A mutagenous effect of the killevir substance and preparation (injectional form) was studied on the standard test-strains of the Salmonella typhimurium TA98, TA100, TA1537, TA1950 and TA1534 in the killevir preparation substance (a fullerenepolyaminocaproic acid) and injectional form do not possess of a mutagenous effect on the Salmonella typhimurium indicator strains.

REFERENCES

1. Methodical instructions on assessment of the pharmacologic substance mutagenous properties // Manual on experimental (preclinical) study of the new pharmacologic substances. – M.: RF MPH. – 2000. – P. 47-61.
2. Statistical treatment of the preclinical test results // Manual on experimental (preclinical) study of the new pharmacologic substances. – M.: RF MPH. – 2000. – P. 360-398.
3. Bochkov N.P., Shram R.Ya., Kuleshov N.N., Zhurkov V.S. System of the chemical substance assessment on a mutageneity for a human; General principles, practical recommendations and further elaborations // Genetics. – 1975. – V. 11. - №10. – P. 156-168.
4. Detection of the chemical compound mutageneity (genetic screening) in the laboratory mice (methodical instructions). – M.: Medicine. – 1977.
5. Preston R.J., Dean B.J., Galloway S., Holden H., mcFee A.F., and Shelby M. Mammalian In Vivo Cytogenetic Aberrations in Bone Marrow Cells // Mutation Research. №189. – 1987. – P. 157-165.
6. §799.9538 TSCA mammalian bone marrow chromosomal aberration test – June 30, 1999 – www.setonresourcecenter.com/cfr/40CFR/P799_056.HTM.
7. Sanitary rules on arrangement, equipping and maintenance of the experimentally-biological clinics (vivaria). – M.: RF MPH. – 1973.