State Federal Enterprise for Science
"Research Centre for Toxicology and Hygienic Regulation of Biopreparations"
at Federal Medico-Biological Agency
(SFES RCT&HRB of FMBA of RF)

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REPORT

on pharmacokinetic study of Killevir therapeutic forms

(Contract # CT-14/2005)

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Serpukhov 2005

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ABBREVIATIONS

AUC – area under pharmacokinetic curve “concentration (C)– time (t) ”;
AUMC - area under pharmacokinetic curve “time derivative multiplied by preparation concentration (C x t)– time (t)”;
C_{max} – maximal concentration of substance (estimated value), ng/ml, ng/g;
T_{max} - time to obtain C_{max} (estimated value), hour;
V_{ss} - quasi-stationary volume of distribution, ml;
MRT - average time of preparation retain in organism, min;
MRT_{rec} - average time of preparation retain in organism at rectal administration, hour;
MRT_{iv} - average time of preparation retain in organism at i/v administration, hour;
Cl – total clearance, ml/h;
D - administered dose, ng;
t – time, min, hour;
ng – nanogram;
ml – milliliter;
i/v – intravenously;
rect. – rectally;
aver. - average value;
st. dev.- standard deviation at 95% significance level.

INTRODUCTION

Key words: *Killevir preparation, pharmacokinetics, intravenous administration, rectal administration, rats, rabbits*.

Report: pages – 45, tables -41, figures -33, References – 12 items.

Objective of the research– study of pharmacokinetics of Killevir preparation therapeutic forms.

The preparation is a derivative from fulleren and aminocapronic acid. This compound has a wide spectrum of applications as a therapeutical, mainly as anti-herpetic preparation.

1. MATERIALS AND METHODS

The studies were performed at Research Center for Toxicology and Hygienic Regulation of Biopreparations, Ministry of Health of the Russian Federation, in accordance with "Methodical recommendations on pre-clinical study of drugs pharmacokinetics" [1].

The experiments were conducted on:

- outbred male rats with mass 280-350 g and
- male Chinchilla rabbits with mass 2.0 – 2.5 kg.

The rats were purchased at Laboratory Animals Breeding Center of RAMS ("Krukovo" branch), and Chinchilla rabbits – at "Manikhino" Animal Breeding Center.

The animals were housed in standard cages under conditions of 12-hour daylight and free access to water and feed.

The animals were handled in compliance with Regulations on Organization, Equipping and Maintaining of Experimentally-Biologic Clinics (vivriums) approved by Ministry of Health of the USSR on July 6, 1973 and fed with natural and cubed feed in accordance with the norms approved by the MH USSR, Order #755, 12.08.77. Before the experiments the animals had passed the quarantine period and adaptation to vivarium conditions for 14 days.

Quantitative determination of Killevir preparation concentration was performed using radioisotope method. For that, a part of hydrogen atoms in molecule of fulleren-polyaminocapronic acid were preliminarily substituted by method of 3-phase catalysis with ^{3}H (tritium) atoms. As a result, a preparation with the following characteristics was produced by the customer for testing: (^{3}H) Killevir solution in dimethylsulfoxide (DMSO) with activity concentration 1mCu/ml and molar radioactivity 600 mCu/mol, concentration of labeled preparation in solution made up 25 mg/ml.

1.1. Determination of Killevir preparation in biological samples

Pharmacokinetics and distribution of the preparation throughout rats' organs was studied for 24 hours after single i/v administration of (^{3}H) Killevir in doses 0.7 and 3.5 mg/kg and rectally in dose 2.8 mg/kg.

At attempts to have the picture of the preparation distribution when rectally administered in the recommended therapeutic dose (0.14 mg/kg), non-interpretative results were obtained. Activity values of the majority of organs (tissues) samples did not considerably differ from background values. Obviously, specific activity of the test preparation was insufficient for testing dose 0.14 mg/kg taking into account bioavailability for the given route of administration.

Renal clearance was assessed for 4 days. Animals were kept in a special cage for taking urine and solid excrements separately with definite time intervals.

The following pharmacokintetic properties of Killevir were examined:

- Distribution of (^{3}H) Killevir in blood and organs after single i/v administration;
- Distribution of (^{3}H) Killevir in blood and organs after single rectal administration;

– liver excretion of (^{3}H) Killevir after single i/v and rectal administration**.**

For experiments on rats at i/v administration Killevir solution in 3% DMSO was prepared so that the dose-to be-administered was contained in 0.15-0.2 ml. The preparation was injected in rat caudal vein. For rectal administration of 0.25 ml, original preparation with concentration 25 mg/ml was added in 2.0 ml of warmed up suppository oil, mixed carefully and cooled down to 35 ^{0}C. For administration of the preparation a disposal syringe and bulb-guard needle was used.

After a definite period of time following administration the animals were sacrificed in CO_2, and samples of blood and organs were taken for examination**.**

Pharmacokintetics of the preparation studied on rabbits for 24 hours after single i/v administration of (^{3}H) Killevir in dose 3.5 mg/kg and rectally in dose 2.8 mg/kg. Assessment of liver clearance was performed for 4 days. Animals were kept in a special cage for taking urine and solid excrements separately with definite time intervals. Time intervals were 5, 10, 15, 30, 60, 120, 240, 360, 960, 1200 and 1440 minutes.

The following pharmacokintetic properties of Killevir were studied:

- Distribution of (^{3}H) Killevir in blood and organs after single i/v administration;
- Distribution of (^{3}H) Killevir in blood and organs after single rectal administration;
- liver excretion of (^{3}H) Killevir after single i/v and rectal administration.

In experiments on rabbits, i/v (in auricular vein) and rectal routes of administration were used. At i/v administration the original Killevir preparation was taken, for rectal administration 0.2 ml of original preparation in concentration 25 mg/ml were added to 0.7 ml of warmed up suppository oil, carefully mixed and cooled down to 35 ^{0}C. After definite time intervals after administering cuts were done on marginal auricular vein to take blood samples of approximately 0.2 ml. Time intervals were similar to those used in experiments on rats.

In both cases the volume of administered preparation was correlated with mass of a separate laboratory animal.

Tissues and organs for the preparation distribution study were selected taking into account the degree of vascularization, declared purpose of the preparation and main eliminating organs.

In addition to blood and urine, the content of Killevir preparation in liver, spleen, kidney, lungs, muscles, epiploon and brain was determined.

A part of animal organ (tissue) (~ 0.2 g) was weighted using laboratory balance Explorer Pro EP214C (Ohaus, Switzerland) and samples were prepared in accordance with the procedure described in 2.2. Radioactivity of samples of plasma, urine and organs taken from animals was measured on liquid scintillation counter Triathler 425-034 (Hidex, Finland) [2]. Scintillation liquid contained PPO solution (2,5- phenyloxasol) and POPOP (1,4-bis 5- phenyloxasolyl benzol) in toluene 5 g/l and 0.1 g/l, respectively. In addition, scintillation liquid contained 30% v/v triton-X100. Calculation effectiveness in conditions of the given experiment was 44-48%.

1.2. Preparation of samples

In the glass volumetric flasks of 10cm^3 ≈ 200 mg of tissue (blood) sample were placed and fixed and then 0.6 ml of acids $HClO_4$(к.) + HNO_3(к.) mixture 1:1 were added. After that, the flasks were covered with “Parafilm”. Prepared flasks were placed above bain-marie, water temperature ≈ 70^{0}C, and kept up to complete dissolving of organs (tissues). Then the flasks were cooled and the content volume was brought to 1 ml with above-mentioned acid mixture. In case blood samples were prepared after initial warming up for 1 hour, 0.1 ml of 30% hydrogen peroxide were added and samples were warmed again up to discoloration. Then the sample volume was brought to 1 ml with acid mixture.

Urine samples were prepared as follows:

- urine from the tray-collector of liquid excrements was washed with 50 ml of 3% water solution of DMSO added with 0.1% triton-X100;
- the obtained solution was evaporated in glass on electric range to 20 ml;
- 10 ml of acid mixture $HClO_4$(conc.) + HNO_3(conc.) was added;
- the obtained mixture was evaporated to 10 ml.

If the solution was not discolored, it was cooled and added with 10 ml of 30% hydrogen peroxide;

- the obtained mixture was also evaporated to 10 ml.

From the obtained samples 25 μl were taken and added to 10 ml of scintillator in scintillating flask; the flask was shaken and kept for 2-3 hours in a cool place.

Now the samples were prepared for measurements on a scintillation counter.

1.3. Calibration

To shift from the measured radioactivity to concentration of the preparation in organs (tissues), 2 calibration diagrams were built:

- radioactivity of initial labeled Killevir preparation depending on its concentration in scintillator volume (Table 1, Fig.1) in concentration range 0.1-500 ng/ml. Calculated by regression method calibration diagram (Table 1, Fig.1) in range 0.1-500 ng/ml is described by linear equation:

$$y=746.45x-4.3 \quad (1)$$

- radioactivity of labeled Killevir preparation as a part of mineralizers of control animals' organs (tissues) depending on its concentration in scintillator volume (Table 2, Fig.2). Conditions of calibration samples preparation and measurement of their radioactivity were similar to those used at measurements of radioactivity in biosamples. The calculated by regression method calibration diagram (Table 2, Fig.2) in range 0.1-100 ng/ml is described by linear equation:

$$y=77.3x+82.6 \quad (2)$$

where: y – number of impulses per minute; x – concentration of Killevir preparation, ng/ml.

Additionally conducted experiments demonstrated that for the majority of organs (tissues) optimal calculation level/introduced activity ratio was observed at administering 25 μl of sample per 10 ml of scintillator which was used in the further experimental studies.

Concentration of the preparation in a sample was determined using ratio (2) of the diagram based on measurements of radioactivity and mass of the test samples.

Table 1

Dependence of standard solutions radioactivity on concentration of the labeled Killevir preparation

Concentration. ng/ml	Radioactivity. (number of impulses per minute)	
	Average	Standard deviation
0.1	82	2
1	1003	12
7.5	2240	21
15	6276	43
30	24584	139
60	44841	149
125	109459	2624
250	186651	3905
500	353524	7298

Dependence of standard solutions radioactivity on concentration of labeled Killevir preparation

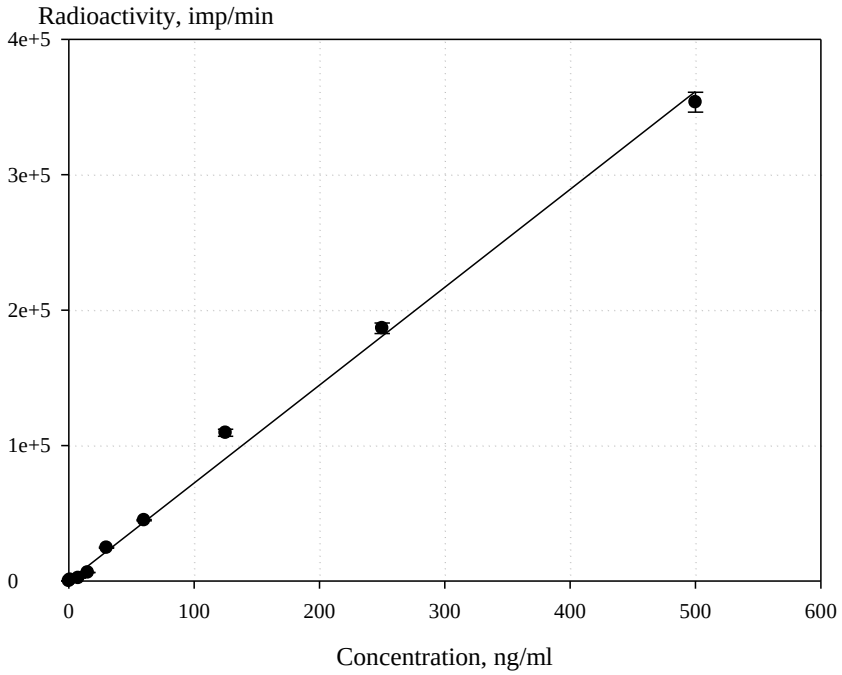

Fig.1.

Table 2

Dependence of radioactivity of solutions containing mineralization of organs and tissues on concentration of the labeled Killevir preparation

Concentration, ng/ml	Radioactivity (number of impulses per minute)	
	Average	Standard deviation
0.1	42	7
1	104	16
2.5	267	28
5	476	60
10	887	91
25	2248	252
50	4463	469
100	7693	907

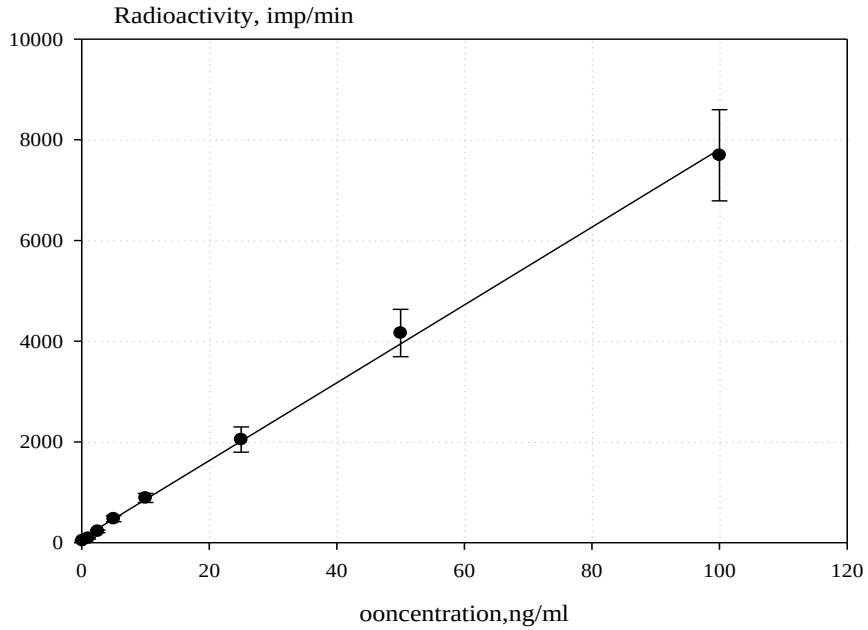

Fig.2.

1.4. Analysis of pharmacokinetic data

Estimation of integral parameters of the preparation pharmacokinetics was performed based on experimentally determined time series of concentrations C=C(t) in blood plasma. In accordance with recommendations [1,4,5] the estimation included calculation of the basic pharmacokinetic parameters AUC and AUMC.

Based on the estimated values of AUC and AUMC areas under pharmacokintetic curves at definite dose D of administered preparation, the following pharmacokintetic parameters were determined:

$$MRT = AUMC / AUC$$

$$Cl = D / AUC$$

$$V_{ss} = Cl \times MRT$$

To analyze experimental data (selection of approximation models), "SigmaPlot" and "TableCurve 2D" programs were used and statistic analysis was carried out in Excel program. Calculation of AUC and AUMC was performed using Advanced Grafer program or numerical integration (Excel).

After selection of pharmacokinetic models for one or other profile, C_{max} and T_{max} were calculated.

2.1. Study of Killevir preparation pharmacokinetics in experiments on rats

Background values of activity were obtained from study of control animals' blood samples.

2.1.1. Study of the preparation pharmacokinetics in experiments on rats at i/v administration in dose 0.7 mg/kg

Values of the preparation concentration in organs and tissues found after different time intervals following i/v administering of the preparation in dose 0.7 mg/kg are presented in Tables 3-10; in Fig.3-10 –graphical presentation.

Table 3

Time-based change of the preparation concentration in blood (ng/ml) after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/ml) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	504.3	773.9	1105.0	816.3	526.8	362.9	341.4	269.0	264.6	203.8	221.6
2	448.2	519.2	1091.5	1063.1	513.5	393.6	395.8	290.0	266.6	184.2	165.7
3	383.5	675.1	1289.8	974.9	640.1	308.1	222.9	176.3	289.5	293.3	258.4
4	219.3	626.6	1096.7	935.5	440.0	487.2	339.7	338.6	173.3	249.3	239.4
average	388.8	648.7	1145.8	947.5	530.1	388.0	324.9	268.5	248.5	232.7	221.3
St.deviation	123.3	105.9	96.2	102.4	82.7	75.0	72.8	68.0	51.4	48.8	40.0

Table 4

Time-based change of the preparation concentration (ng/g) in spleen after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	682.2	724.9	1335.3	1369.6	898.9	654.0	952.4	487.6	498.8	362.2	404.8
2	452.1	519.8	1084.4	1422.2	674.4	465.8	620.3	662.2	411.5	487.2	426.1
3	443.4	692.8	1467.1	1162.4	776.8	742.8	896.1	642.9	536.7	501.7	558.6
4	473.3	833.4	1370.7	1470.1	681.7	680.5	758.6	504.7	406.0	582.8	479.7
average	512.8	692.7	1314.4	1356.1	757.9	635.8	806.9	574.3	463.3	483.5	467.3
St.deviation	113.7	130.0	163.1	135.5	104.9	119.3	148.6	90.9	64.8	91.1	68.5

Table 5

Time-based change of the preparation concentration (ng/g) in epiploon after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	289.6	741.1	859.7	449.0	466.5	160.7	249.6	176.2	204.2	65.5	50.9
2	368.6	627.3	890.1	672.3	332.6	255.5	157.1	212.0	121.5	119.0	99.8
3	353.7	654.3	1099.6	693.5	276.2	332.1	138.8	86.8	148.4	118.3	146.1
4	550.5	862.1	1034.0	514.5	360.2	264.7	321.0	46.9	41.2	192.8	95.6
average	390.6	721.2	970.8	582.3	358.9	253.3	216.6	130.5	128.8	123.9	98.1
St.deviation	112.0	105.7	114.6	119.5	79.8	70.5	84.8	76.7	67.8	52.3	38.9

Table 6

Time-based change of the preparation concentration (ng/g) in kidney after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	525.5	988.0	479.0	1048.4	851.2	920.5	789.7	860.1	853.4	850.4	802.8
2	634.7	831.4	779.9	909.9	786.4	931.6	943.6	839.2	883.7	875.0	690.1
3	511.5	675.3	745.6	988.3	832.2	923.5	629.9	785.1	673.9	825.5	924.3
4	479.4	813.3	807.6	787.0	1081.9	712.4	711.8	649.8	762.8	692.1	808.3
average	537.8	827.0	703.0	933.4	887.9	872.0	768.7	783.5	793.4	810.7	806.3
St.deviation	67.4	128.0	151.5	112.9	132.1	106.5	133.6	94.6	94.9	81.6	95.7

Table 7

Time-based change of the preparation concentration (ng/g) in liver after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	588.1	752.4	818.7	1049.1	738.9	479.1	740.3	606.8	582.1	550.9	397.0
2	349.4	901.7	876.8	986.2	608.0	544.5	567.2	658.8	424.2	492.9	458.6
3	294.1	867.1	622.9	1223.9	825.7	571.0	598.8	457.2	436.1	490.7	511.0
4	398.6	873.0	704.9	1330.2	1038.7	333.7	675.5	626.0	323.1	292.3	364.9
average	407.5	848.5	755.8	1147.3	802.8	482.1	645.4	587.2	441.4	456.7	432.9
St.deviation	127.7	65.8	113.8	158.0	180.9	106.2	77.9	89.3	106.6	113.1	65.0

Table 8

Time-based change of the preparation concentration (ng/g) in muscles tissue after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	253.8	136.8	376.1	296.6	373.7	165.6	96.9	257.4	214.9	152.9	52.3
2	169.6	235.6	299.3	429.9	334.1	107.8	251.8	288.8	142.6	45.2	118.8
3	239.9	109.4	525.1	451.3	309.1	96.4	211.5	179.2	166.1	72.1	73.9
4	227.4	220.5	443.9	291.5	91.7	210.2	90.9	144.3	72.3	104.4	104.2
average	222.7	175.6	411.1	367.3	277.1	145.0	162.8	217.4	149.0	93.6	87.3
St.deviation	37.0	61.9	96.3	85.1	126.5	53.0	81.3	67.1	59.3	46.3	29.9

Table 9

Time-based change of the preparation concentration (ng/ml) in brain tissue after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	312.6	416.3	552.0	263.2	245.1	207.5	218.8	175.7	240.5	275.5	239.9
2	156.1	431.1	435.4	259.5	208.5	305.1	304.2	164.5	289.8	193.8	170.4
3	418.9	376.6	299.3	203.8	246.3	159.3	118.9	286.9	140.8	160.7	183.4
4	304.2	236.1	417.3	153.0	421.1	137.9	160.4	210.5	201.8	95.6	254.5
average	297.9	365.0	426.0	219.9	280.3	202.5	200.6	209.4	218.2	181.4	212.0
St.deviation	108.0	89.0	103.4	52.2	95.5	74.4	80.3	55.3	63.0	74.8	41.4

Table 10

Time-based change of the preparation concentration (ng/g) in lungs tissue after i/v administration in rats in dose 0.7 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	963.6	1401.1	1128.3	419.1	623.9	563.3	473.1	511.3	511.4	323.7	255.4
2	1248.5	1400.9	905.1	700.6	504.8	418.5	379.9	560.4	359.2	153.4	294.4
3	1135.9	1311.8	924.4	669.5	671.7	568.8	532.8	580.2	301.9	316.6	129.6
4	1078.1	1131.7	805.3	719.7	742.4	499.0	520.8	385.8	417.9	273.9	269.7
average	1106.5	1311.4	940.8	627.2	635.7	512.4	476.7	509.4	397.6	266.9	237.3
St.deviation	118.7	127.0	135.5	140.3	99.9	70.2	69.5	87.4	89.5	78.8	73.6

Analysis of obtained data demonstrated that time-based change of Killevir preparation concentration in organs, tissues and blood after i/v administration in rats is quite satisfactorily described by 2-part model with adsorption [4]:

$$C_t = C_1 * \exp(-k_\alpha t) + C_2 * \exp(-k_\beta t) - C_3 * \exp(-k_{ab} t)$$
$$C_3 = C_1 + C_2 \qquad (3)$$

where: k_α , k_β – coefficients of elimination for different phases; k_{ab} -coefficient of adsorption. Values of coefficients of elimination and adsorption, pharmacokintetic parameters of AUC, AUMC, MRT, MRT, T_{max} and C_{max} in test organs are presented in Table 11. At the same time, it should be mentioned that this type of approximation (3) is not an exhaustive optimal since it can not explain evident "dips" in kinetics of curves for some organs (e.g., spleen, liver, etc.) in time range 1-4 hours. This effect is more pronounced at administering 5-fold therapeutic doses (3.5 mg/kg), (see Tables and diagrams below).

Time-based change of the Killevir preparation concentration in rats' blood after i/v administration in dose 0.7 mg/kg

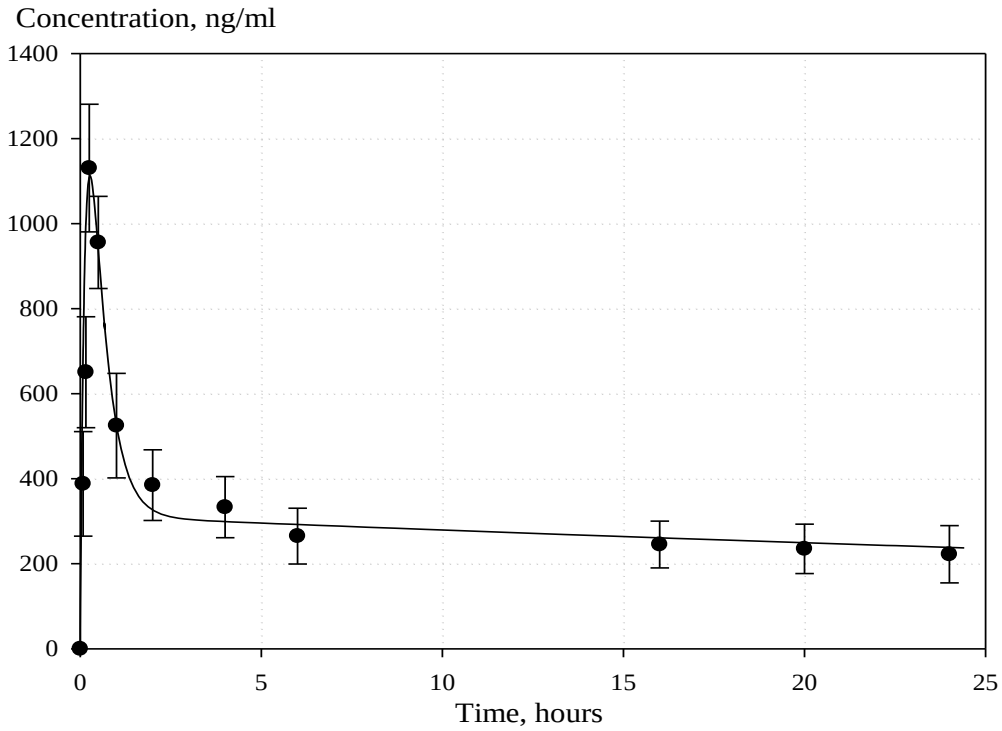

Fig.3.

Time-based change of the Killevir preparation concentration in rats' blood after i/v administration in dose 0.7 mg/kg

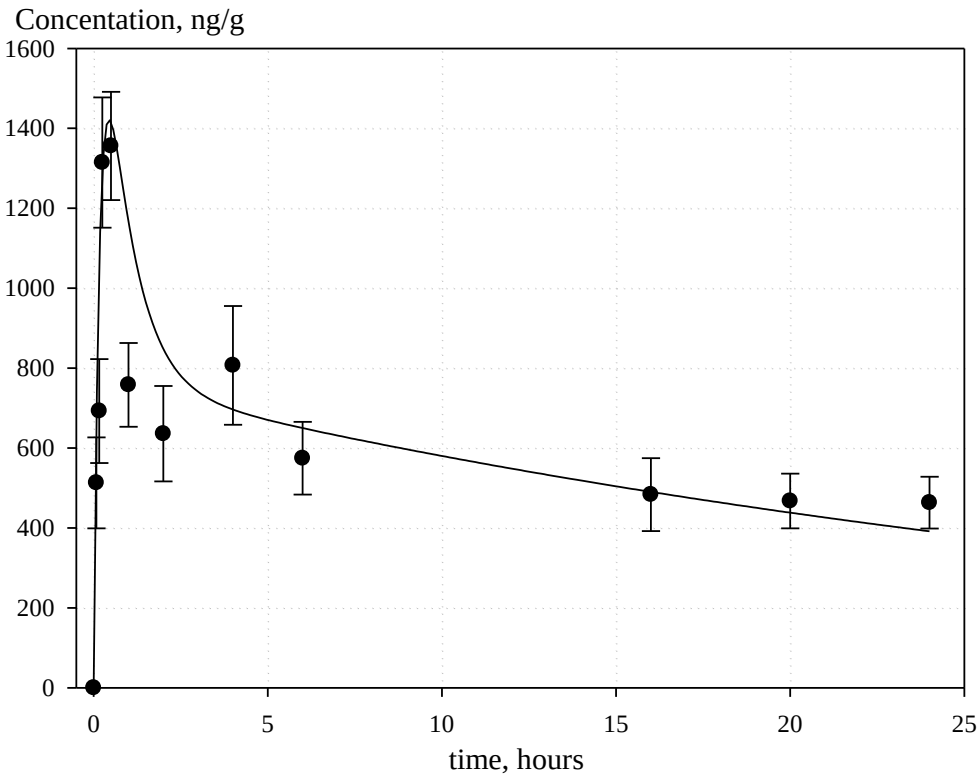

Fig.4

Time-based change of the Killevir preparation concentration in rats' epiploon after i/v administration in dose 0.7 mg/kg

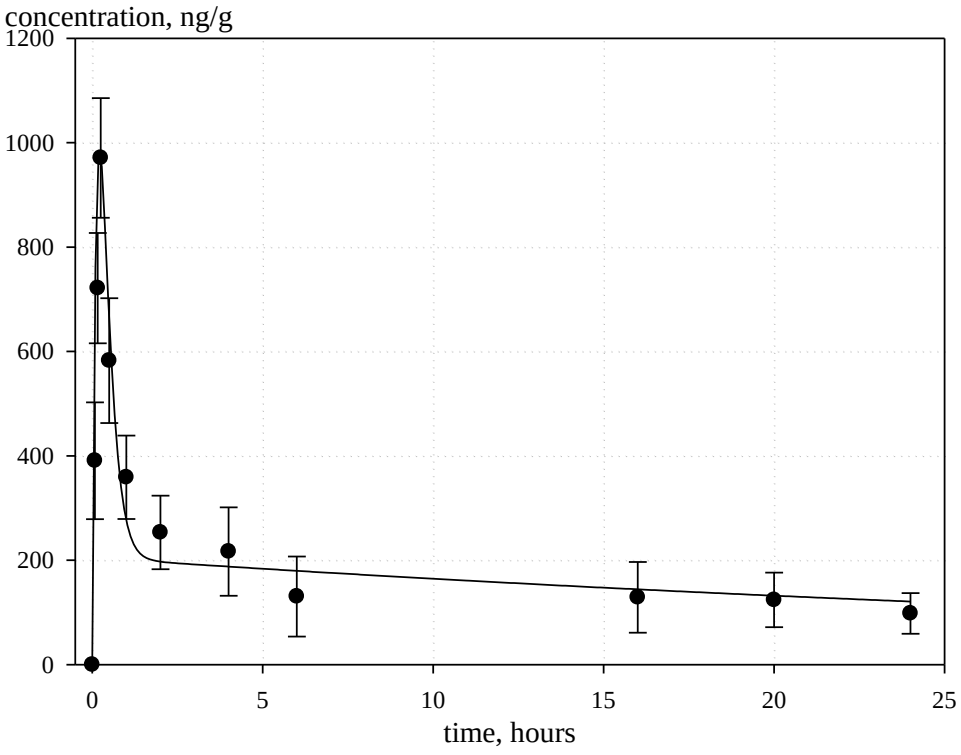

Fig.5

Time-based change of the Killevir preparation concentration in rats' kidney after i/v administration in dose 0.7 mg/kg

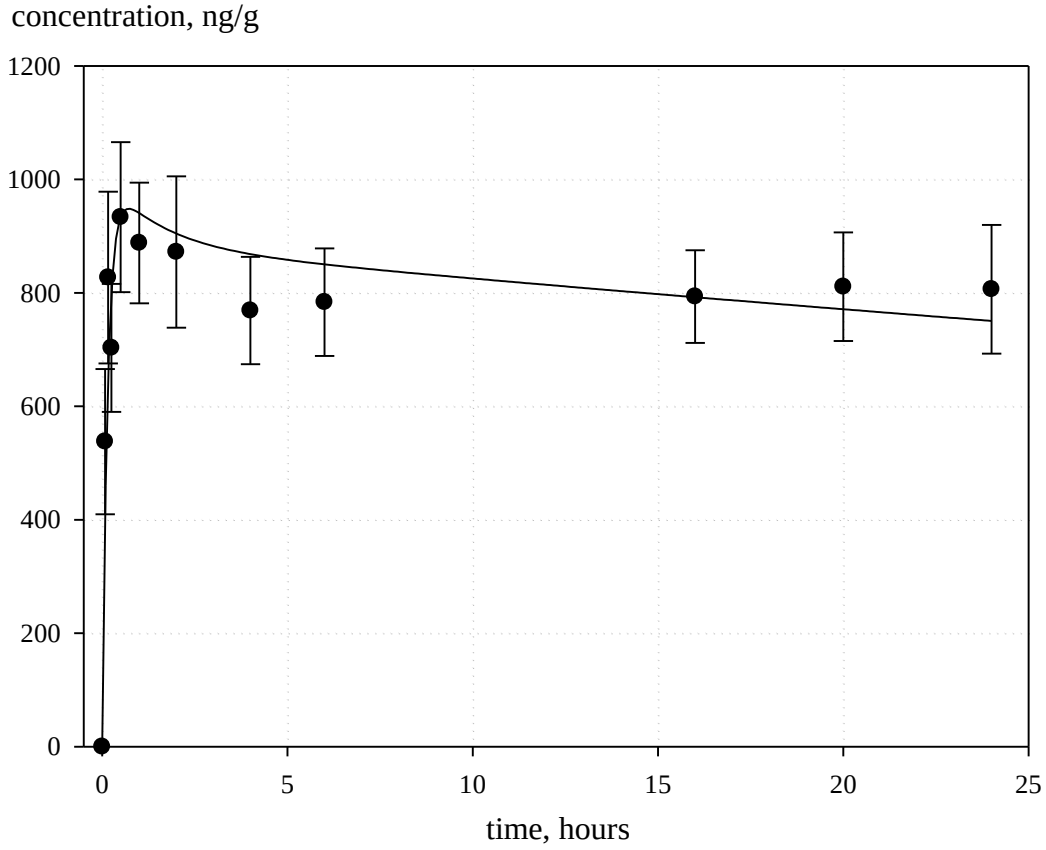

Fig.6

Time-based change in Killevir preparation concentration in rats' liver after i/v administration in dose 0.7 mg/kg

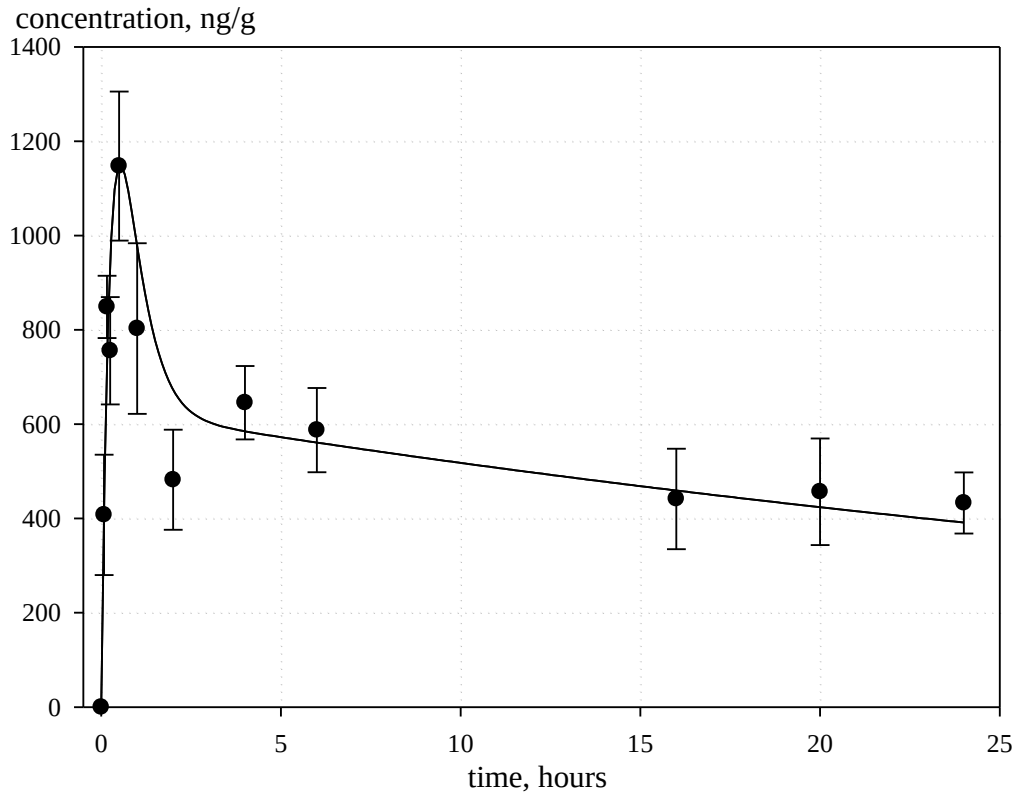

Fig.7

Time-based change of the in Killevir preparation concentration in rats' muscles tissue after i/v administration in dose 0.7 mg/kg

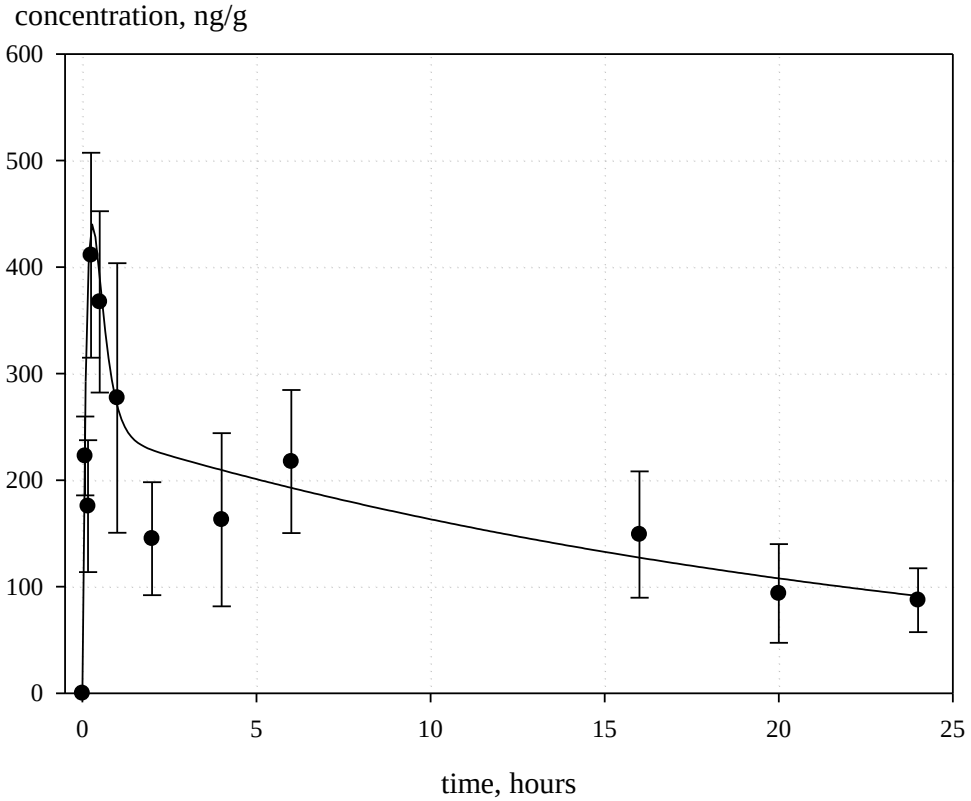

Fig.8

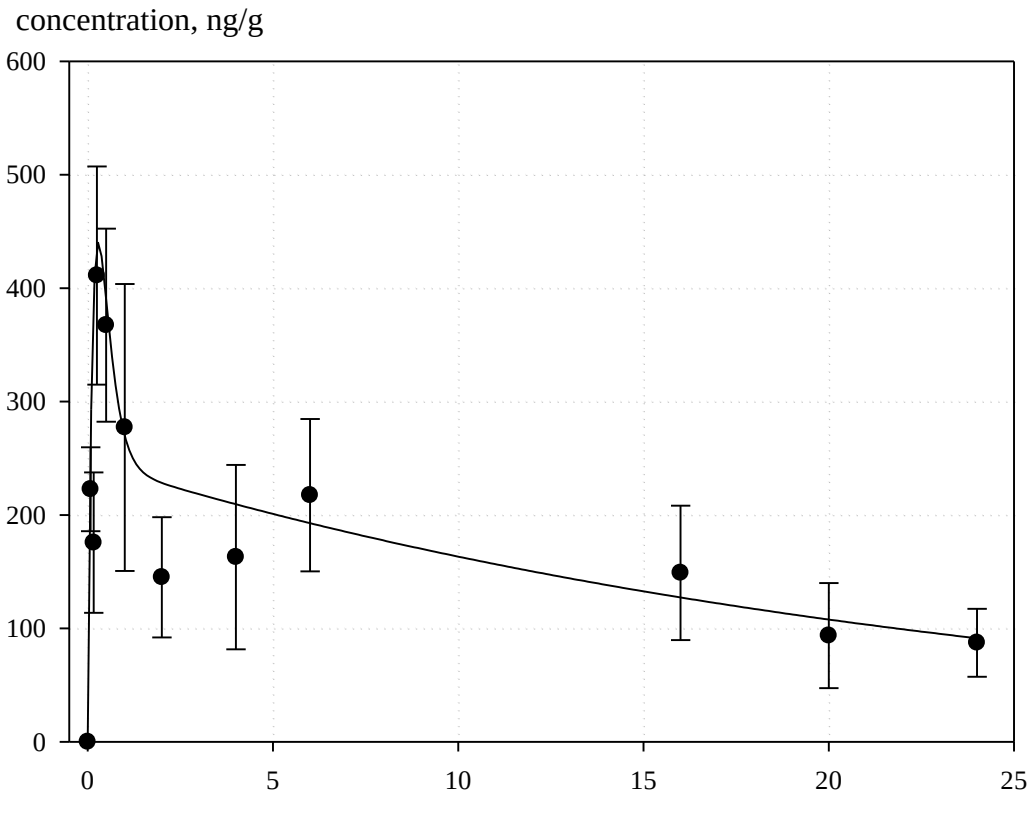

Fig.9

Time-based change of the Killevir preparation concentration in rats' lungs tissue after i/v administration in dose 0.7 mg/kg

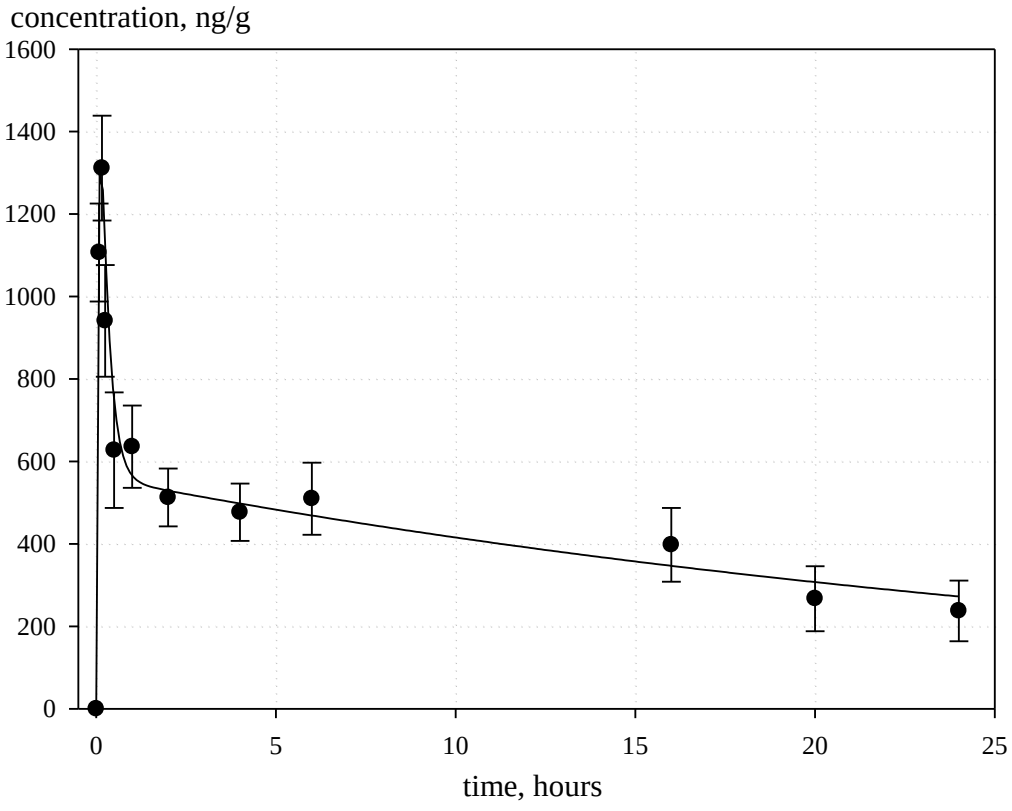

Fig.10

Table 11

Characteristics of the Killevir preparation distribution in rats' organs at i/v administration in dose 0.7 mg/kg

Test organ	AUC (ng/ml)*hour	AUMC (ng/ml)*hour^2	MRT hour	k_α / k_β .hour $^{-1}$	k_{ab} . hour $^{-1}$	Tmax. hour	Cmax. ng/ml
Blood	7169.8	75697.8	10.56	2.375/0.0113	6.595	0.271	1112.5
Lungs	9864.9	101438.3	10.28	4.495/0.0302	17.340	0.126	1340.0
Liver	12596.2	133678.4	10.61	2.606/0.0202	2.336	0.531	1149.8
Spleen	14198.9	143917.7	10.14	5.468/0.0281	1.257	0.442	1422.1
Kidney	19598.5	228634.9	11.67	0.662/0.0068	6.651	0.715	948.3
Muscles	3866.1	37785.4	9.77	4.777/0.0415	4.374	0.289	440.1
Epiploon	4241.2	41965.6	9.89	5.207/0.0221	4.848	0.216	988.4
Brain	5401.6	60381.6	11.18	3.153/0.0135	11.435	0.207	438.4

2.1.2. Study of the preparation pharmacokinetics in experiments on rats at i/v administration in dose 3.5 mg/kg

The values of the preparation concentrations in organs and tissues studied in different periods of time after i/v administration of the preparation in dose of 3.5 mg/kg are given in Tables 12-19, and the same data is presented graphically in Fig.11-18.

Table 12

Time-based change of the preparation concentration (ng/ml) in blood after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/ml) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	960.3	895.3	580.5	948.5	975.6	762.5	470.7	485.2	494.5	454.3	370.8
2	1047.0	706.3	594.5	799.1	937.3	831.8	472.1	315.1	396.8	391.9	467.0
3	1205.8	792.5	610.9	801.1	935.1	884.8	620.8	457.1	405.2	391.3	366.5
4	996.9	712.4	712.0	717.1	744.4	737.2	509.8	431.8	347.6	467.9	457.0
average	1052.5	776.6	624.5	816.5	898.1	804.1	518.3	422.3	411.0	426.4	415.3
St.deviation	108.2	88.3	59.6	96.4	104.2	67.0	70.7	74.7	61.2	40.5	54.1

Table 13

Time-based change of the preparation concentration (ng/g) in spleen after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals. (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	1074.2	649.8	588.7	1157.0	1489.0	1230.6	869.7	888.6	623.4	619.7	673.1
2	878.9	797.8	628.4	1319.0	1426.6	931.2	833.3	777.2	721.6	652.9	734.2
3	894.4	660.0	484.1	1130.9	1484.7	1067.7	819.0	824.6	798.0	680.6	608.9
4	763.5	782.5	613.1	1006.8	1802.8	940.9	1009.9	764.1	730.1	766.6	649.9
average	902.7	722.5	578.6	1153.4	1550.8	1042.6	883.0	813.6	718.3	679.9	666.5
St.deviation	128.4	78.4	65.1	128.4	170.4	139.9	87.3	56.3	71.9	62.9	52.3

Table 14

Time-based change of the preparation concentration (ng/g) in epiploon after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	511.6	372.6	377.1	630.3	750.4	433.4	493.1	432.2	273.4	300.0	212.0
2	538.5	505.6	250.7	660.9	619.4	532.3	400.5	366.9	370.0	231.6	293.0
3	444.6	415.8	414.0	509.4	679.1	570.4	408.2	452.4	323.9	291.9	199.0
4	414.6	476.4	397.7	688.9	623.6	415.1	347.7	378.7	254.2	255.7	136.9
average	477.4	442.6	359.9	622.4	668.1	487.8	412.4	407.5	305.4	269.8	210.2
St.deviation	57.5	59.8	74.3	79.0	61.2	75.4	60.2	41.2	52.2	32.0	64.2

Table 15

Time-based change of the preparation concentration (ng/g) in kidney after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	2219.9	1457.9	436.8	1839.4	2239.3	1672.0	1412.8	1560.2	1078.3	882.0	1080.4
2	2033.3	1499.5	766.4	1726.4	2075.1	1474.5	1413.2	1345.8	1228.0	1078.5	961.6
3	2291.0	1428.7	556.6	1622.6	2045.2	1672.1	1061.7	1381.4	1169.5	934.6	962.3
4	2202.8	1663.0	589.0	2041.9	2274.5	1782.9	1377.3	1596.6	1240.6	947.0	997.0
average	2186.8	1512.3	587.2	1807.6	2158.5	1650.4	1316.3	1471.0	1179.1	960.5	1000.3
St.deviation	109.2	104.6	136.2	179.6	115.1	128.4	170.5	125.7	74.0	83.5	55.9

Table 16

Time-based change of the preparation concentration (ng/g) in liver after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	1816.5	1413.2	767.1	1519.2	2793.7	1761.6	1509.0	1132.3	1068.7	1152.6	1124.2
2	2069.1	1349.2	590.6	1643.9	2827.0	2015.5	1447.8	1075.5	1166.4	1199.7	1115.6
3	1861.8	1457.1	616.1	1743.9	2564.8	1853.5	1704.9	1392.2	1229.5	1026.8	994.5
4	2029.2	1311.5	746.6	1502.0	2817.7	1870.9	1677.2	1299.2	1154.3	1059.5	982.0
average	1944.1	1382.8	680.1	1602.2	2750.8	1875.4	1584.7	1224.8	1154.7	1109.6	1054.0
St.deviation	123.7	64.9	89.6	113.7	124.8	105.0	125.8	146.5	66.2	80.3	76.3

Table 17

Time-based change of the preparation concentration (ng/g) in muscles after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	702.2	438.9	256.9	488.7	855.0	569.0	541.4	381.7	415.5	257.0	347.8
2	721.7	639.9	266.2	602.5	750.9	609.3	448.5	406.9	419.3	406.6	224.2
3	856.4	649.0	340.0	443.7	730.3	468.5	478.7	464.9	300.6	305.5	422.2
4	814.2	497.9	366.6	474.4	935.2	423.5	358.1	218.3	398.0	373.7	341.9
average	773.6	556.4	307.4	502.3	817.8	517.5	456.7	368.0	383.3	335.7	334.1
St.deviation	73.7	104.5	54.2	69.4	95.4	86.3	76.3	105.7	55.9	67.3	81.8

Table 18

Time-based change of the preparation concentration (ng/g) in brain tissue after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	982.8	676.9	271.6	677.9	524.9	563.2	521.8	424.1	267.4	220.7	275.1
2	1095.6	761.9	366.8	848.7	761.3	552.5	464.2	265.5	236.4	324.9	308.4
3	1202.0	629.5	293.1	854.5	704.4	456.8	526.7	323.7	298.6	375.0	235.3
4	1279.5	775.1	373.2	703.7	751.3	587.3	412.2	291.4	357.6	196.6	233.3
average	1140.0	710.8	326.2	771.2	685.5	540.0	481.3	326.2	290.0	279.3	263.0
St.deviation	129.0	69.5	51.4	93.5	109.9	57.3	54.1	69.5	51.7	84.7	35.9

Table 19

Time-based change of the preparation concentration (ng/g) in lungs tissue after i/v administration in rats in dose 3.5 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	2065.9	1414.0	1127.6	1517.4	1562.9	722.2	708.5	454.8	485.7	496.6	447.9
2	2159.8	1367.2	1180.2	1819.3	1338.4	753.1	802.7	456.4	605.2	390.6	381.2
3	2221.3	1410.8	920.4	1740.4	1440.8	571.9	885.6	633.1	461.6	519.2	392.1
4	2412.5	1609.4	1228.1	1558.1	1345.7	734.7	690.3	501.2	377.1	486.3	525.0
average	2214.9	1450.3	1114.1	1658.8	1421.9	695.5	771.8	511.4	482.4	473.2	436.6
St.deviation	146.4	108.2	135.5	144.4	104.9	83.3	90.5	83.9	94.2	56.7	65.8

The effect partially revealed in some organs at i/v administration of one therapeutical dose in rats was displayed more evidently at D=3.5 mg/kg. Though the approximation of ratio (3) was suitable at description of previous experiment results, in this case more universal affinity is required. The structure of equation, appearing at approximation of intermitting effect (e.g. in case of periodic injections), where the concentration profile C_t passing through minimum after the previous administration is increased with the next one can belong to such type of affinity.

For description of such bimodal curves the following ratio was used:

$$\tilde{N}_t = \sum_{i=1,3} C_i \times \exp(-k_i t) + \sum_{i=4,6} C_i \times \exp\left[-k_i (t - t_0)\right] \qquad (4)$$

where: k_i – coefficients of elimination (k_α and k_β) and absorption (k_{ab}) for different routes of delivery; t_0 – characteristic "effective" time of secondary effect. The first component in ratio (4) is a kind of transformed record of ratio (3) and is "responsible" for initial phase of distribution, and the second – for the sum of thermal content of the first phase and the effect of the preparation "secondary supply". The values of elimination coefficients, coefficient of absorption, pharmacokinetic parameters AUC, AUMC, MRT, MRT, T_{max} and C_{max} in the studied organs are given in Table 20.

Time-based change in the Killevir preparation concentration in rats' blood after i/v administration in dose 3.5 mg/kg

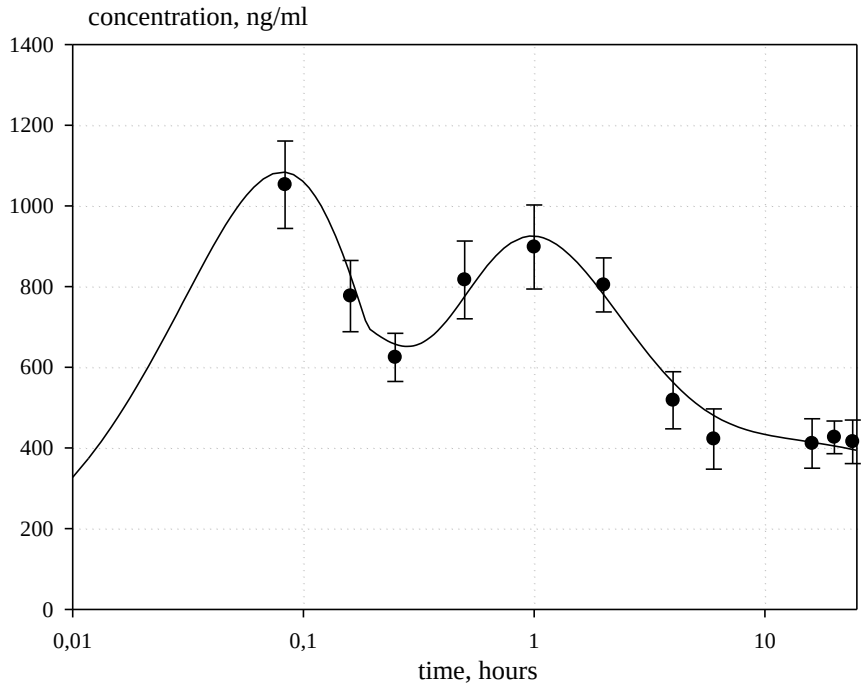

Fig.11.

Time-based change in the Killevir preparation concentration in rats' spleen after i/v administration in dose 3.5 mg/kg

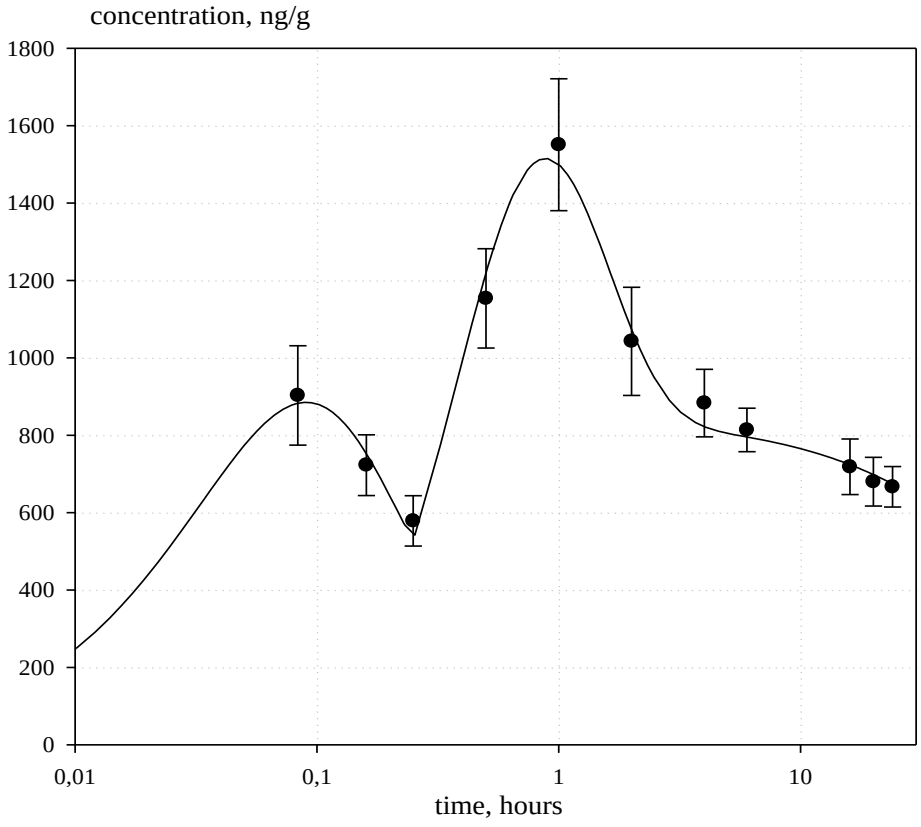

Fig.12

Time-based change in the Killevir preparation concentration in rats' epiploon after i/v administration in dose 3.5 mg/kg

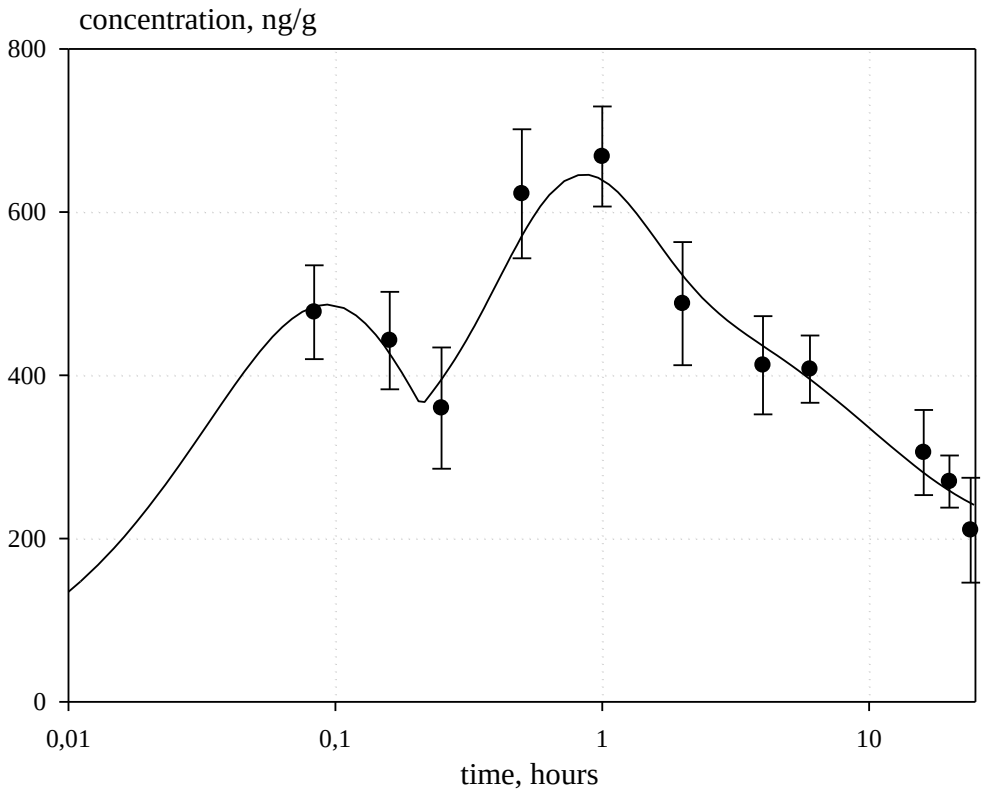

Fig.13

Time-based change in the Killevir preparation concentration in rats' kidney after i/v administration in dose 3.5 mg/kg

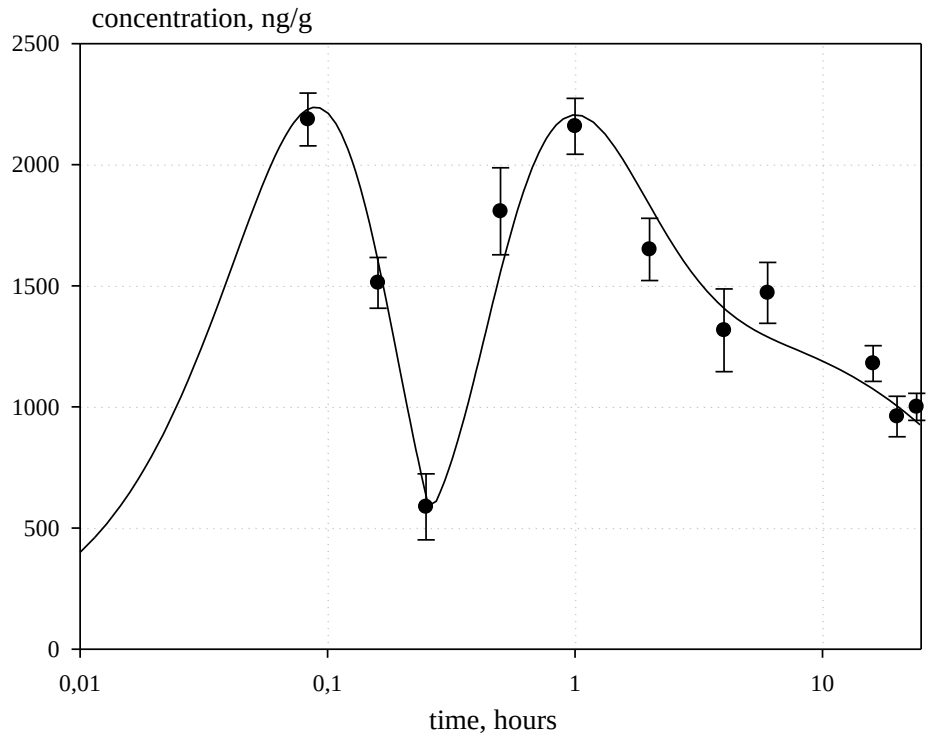

Fig.14

Time-based change of the Killevir preparation concentration in rats' liver after i/v administration in dose 3.5 mg/kg

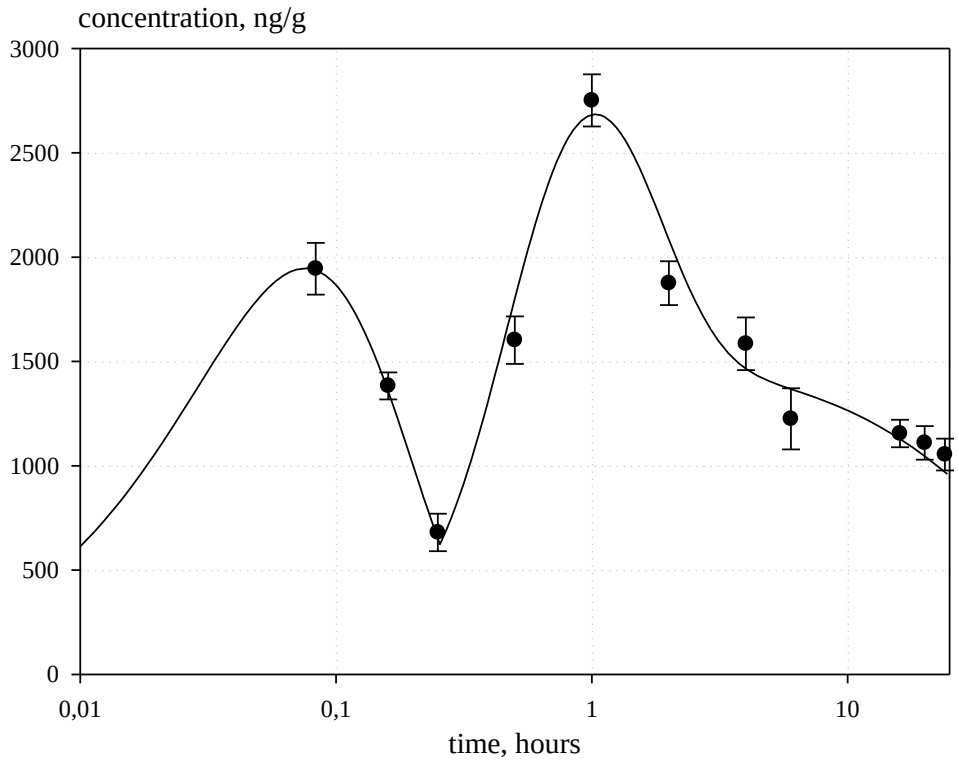

Fig.15

Time-based change in the Killevir preparation concentration in rats' kidney after i/v administration in dose 3.5 mg/kg

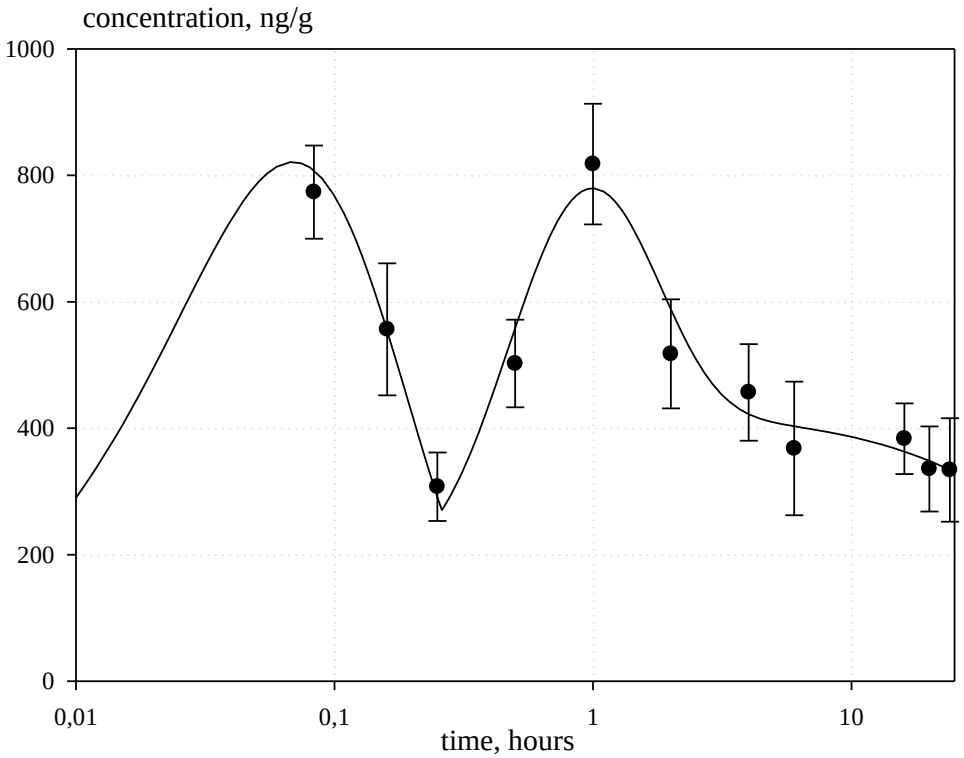

Fig.16

Time-based change in the Killevir preparation concentration in rats' kidney after i/v administration in dose 3.5 mg/kg

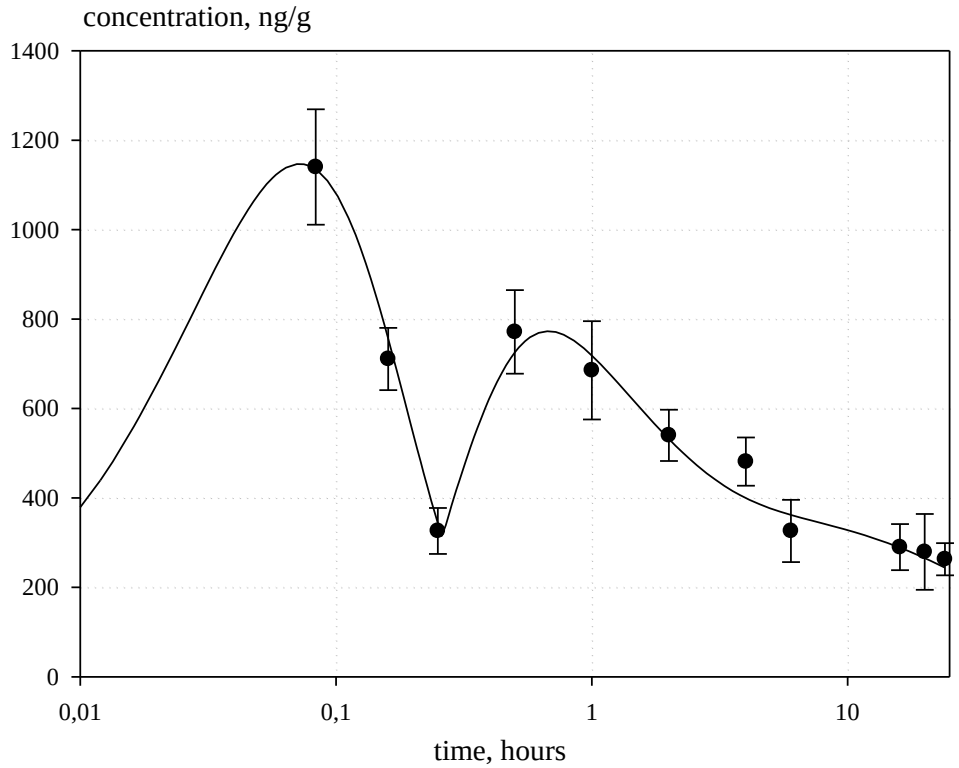

Fig.17

Time-based change in the Killevir preparation concentration in rats' lungs after i/v administration in dose 3.5 mg/kg

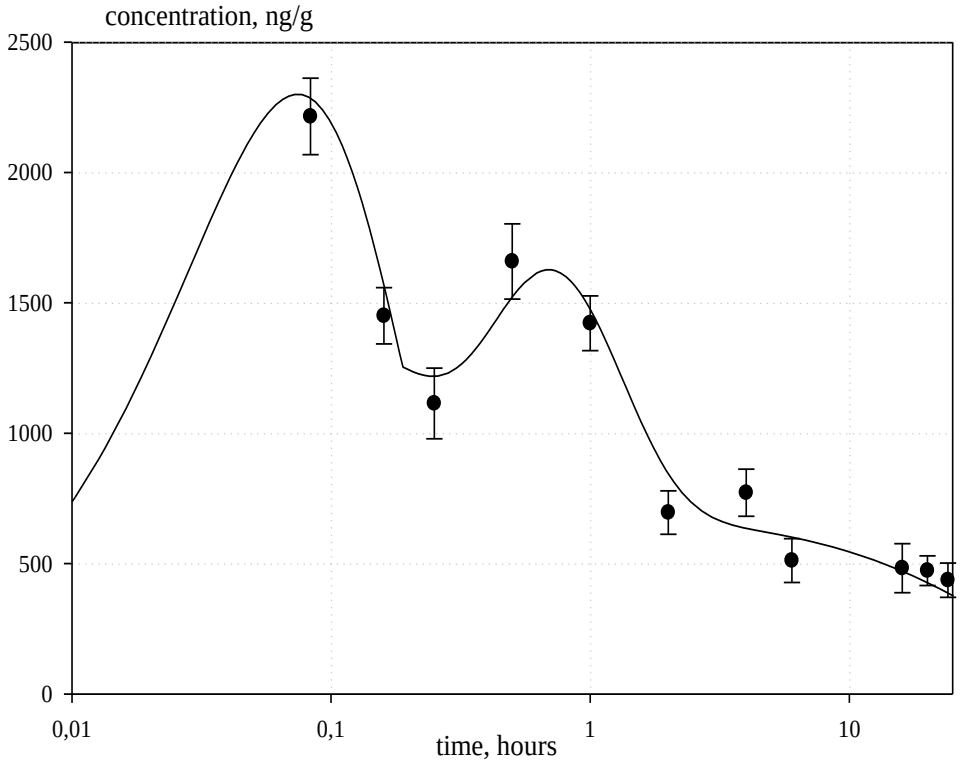

Fig.18

Table 20

Characteristics of the Killevir preparation distribution in rats' organs at i/v administration in dose 3.5 mg/kg

Test organ	AUC (ng/ml)*hour	AUMC (ng /ml)*hour^2	MRT hour	k_β . hour $^{-1\ *)}$	k_{ab} . hour $^{-1\ **)}$	Tmax. (1)/(2) hour	Cmax. (1)/(2) ng/ml (g)
Blood	11915.3	155742.2	13.07	0.517	12.497	0.081/0.951	1083.4/925.4
Lungs	14492.8	162394.8	11.2	2.271	12.543	0.074/0.682	2299.9/1621.0
Liver	31779.7	384776.7	12.11	1.627	11.819	0.076/1.034	1946.4/2684.2
Spleen	19437.1	365413.2	18.80	1.892	12.137	0.091/0.887	885.2/1516.4
Kidney	29979.8	373511.0	12.46	0.829	20.032	0.089/0.982	2237.4/2206.2
Muscles	9561.4	105973.9	11.08	0.010	7.795	0.069/1.021	821.2/779.3
Epiploon	8420.5	121376.9	14.4	0.098	11.997	0.093/0.853	486.7/645.4
Brain	8316.6	94153.7	11.32	0.824	12.384	0.074/0.673	1146.8/770.3

Note.

– $^{*)}$ k_β - for secondary curve;

– $^{**)}$ k_{ab} – for primary curve;

– values T_{max} and C_{max} are given for primary (1) and secondary (2) curves, respectively.

2.1.3. Study of the preparation pharmacokinetics in experiments on rats at rectal administration of dose 2.8 mg/kg

The values of the preparation concentrations in organs and tissues studied in different periods of time after rectal administration of the preparation in dose 2.8 mg/kg are given in Tables 21-28 and the same data is presented graphically in Fig.19-26.

Table 21

Time-based change of the preparation concentration (ng/ml) in blood plasma following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/ml) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	295.8	453.1	440.3	467.3	316.4	312.0	408.0	520.4	440.6	422.1	364.2
2	322.1	322.6	466.1	558.0	493.0	393.0	282.0	496.1	364.3	451.7	448.7
3	192.2	370.9	444.1	585.2	406.9	299.0	225.8	476.6	349.2	348.4	297.5
4	336.1	285.8	298.6	453.7	337.2	236.9	352.4	321.4	499.4	315.4	441.1
average	286.5	358.1	412.3	516.1	388.4	310.2	317.1	453.6	413.4	384.4	387.9
St.deviation	65.1	72.3	76.7	65.3	79.8	64.2	79.7	89.9	69.9	63.3	71.3

Table 22

Time-based change of the preparation concentration (ng/g) in spleen following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	202.0	283.8	274.7	572.5	406.1	296.0	406.9	557.0	383.4	388.1	381.2
2	178.6	310.1	393.7	470.4	464.4	415.6	468.4	764.7	417.0	417.7	313.0
3	200.4	180.2	446.3	478.8	283.5	388.2	456.8	764.4	475.6	314.4	335.2
4	301.7	324.1	296.6	412.8	413.5	297.4	609.8	618.0	544.5	281.4	246.7
average	220.7	274.5	352.9	483.6	391.9	349.3	485.5	676.0	455.1	350.4	319.0
St.deviation	55.1	65.1	81.0	66.1	76.7	61.8	87.1	105.2	70.7	63.3	55.9

Table 23.

Time-based change of the preparation concentration (ng/g) in epiploon following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	88.6	204.4	296.8	386.3	286.9	327.1	263.2	340.1	298.2	221.7	235.9
2	140.5	275.8	267.5	266.0	198.5	252.2	216.9	222.1	382.6	239.4	315.4
3	201.0	278.9	284.0	247.1	318.0	243.9	239.7	372.6	253.0	242.0	189.3
4	204.1	303.8	393.1	324.4	334.7	221.2	154.0	359.6	315.1	336.1	279.3
average	158.6	265.7	310.4	306.0	284.5	261.1	218.5	323.6	312.2	259.8	255.0
St.deviation	55.1	42.8	56.4	62.9	60.7	45.9	46.9	69.0	53.7	51.7	54.5

Table 24

Time-based change of the preparation concentration (ng/g) in kidney following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	162.8	89.5	199.5	338.7	490.7	414.1	281.4	258.7	284.4	265.3	221.7
2	151.4	232.5	231.1	357.0	403.0	342.2	353.7	229.0	334.4	280.9	273.1
3	71.4	133.6	385.7	316.5	315.6	308.1	391.9	249.9	256.1	163.5	171.4
4	102.0	182.1	270.8	232.2	392.9	298.2	232.4	368.1	146.4	214.1	168.0
average	121.9	159.4	271.8	311.1	400.5	340.6	314.9	276.4	255.3	230.9	208.5
St.deviation	42.8	61.7	81.4	55.2	71.7	52.5	71.6	62.4	79.5	53.2	49.5

Table 25

Time-based change of the preparation concentration (ng/g) in liver following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	60.8	158.9	194.3	235.4	335.5	474.5	275.4	489.2	369.6	333.1	355.7
2	26.2	130.9	285.2	360.4	258.7	346.3	313.9	482.6	285.2	365.9	283.3
3	70.5	138.9	179.6	206.3	332.6	438.9	389.5	465.8	261.5	251.6	307.8
4	46.8	54.6	109.9	338.5	469.6	466.0	429.1	336.8	434.7	215.1	210.0
Average	51.1	120.8	192.2	285.2	349.1	431.4	352.0	443.6	337.7	291.4	289.2
St.deviation	19.2	45.7	72.1	75.7	87.8	58.7	69.9	71.9	79.5	70.0	60.8

Table 26

Time-based change of the preparation concentration (ng/g) in muscles following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	146.7	214.4	153.6	471.1	408.1	439.2	354.1	240.4	301.8	261.5	240.8
2	74.0	110.3	211.1	332.7	578.2	299.9	314.5	366.9	245.3	177.5	190.3
3	195.8	254.2	303.8	448.7	482.9	407.7	226.2	296.6	211.9	261.4	346.1
4	89.3	170.9	293.8	452.9	558.6	407.4	221.0	275.7	137.3	298.4	207.8
average	126.4	187.5	240.6	426.4	507.0	388.5	278.9	294.9	224.1	249.7	246.2
St.deviation	55.8	61.7	71.3	63.2	77.7	61.0	66.0	53.3	68.7	51.2	69.8

Table 27

Time-based change of the preparation concentration (ng/g) in brain following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	214.6	195.9	367.0	435.4	340.1	163.0	315.2	361.3	351.7	231.4	171.9
2	121.1	185.1	347.8	390.7	448.5	281.1	212.7	443.7	242.0	244.5	272.4
3	167.4	215.8	280.6	477.7	442.8	236.3	261.5	299.8	347.8	334.2	282.0
4	115.1	301.8	201.1	501.5	498.8	203.7	269.3	444.2	344.1	306.1	201.4
average	154.6	224.7	299.1	451.3	432.6	221.0	264.7	387.3	321.4	279.1	231.9
St.deviation	46.3	53.0	75.1	48.8	66.6	50.1	42.0	70.1	53.0	49.1	53.8

Table 28

Time-based change of the preparation concentration (ng/g) in lungs tissue following rectal administration in rats in dose 2.8 mg/kg

Animal #	Preparation concentration (ng/g) in different time intervals (min)										
	5	10	15	30	60	120	240	360	960	1200	1440
1	186.9	272.3	263.8	505.3	671.9	498.6	442.0	524.8	344.3	294.6	310.3
2	125.6	280.4	236.8	597.9	592.6	392.0	386.7	462.6	293.0	330.3	218.3
3	114.8	179.8	400.6	676.3	723.8	492.2	416.5	560.5	378.3	360.6	215.5
4	73.8	315.8	301.8	527.4	737.6	425.6	274.3	556.5	461.7	235.9	259.7
average	125.3	262.1	300.7	576.7	681.5	452.1	379.9	526.1	369.3	305.4	251.0
St.deviation	46.8	58.0	71.7	77.2	65.7	51.9	73.9	45.3	70.9	53.6	44.4

The approaches previously applied for approximation of experimental data in case of rectal administration were used at describing pharmacokinetical curves after rectal administration of the Killevir preparation suppository in rats. The values of elimination coefficients, coefficient of absorption, pharmacokinetic parameters AUC, AUMC, MRT, MRT, T_{max} and C_{max} in studied organs are given in Table 29.

Time-based change of the Killevir preparation concentration in blood following rectal administration in rats in dose 2.8 mg/kg

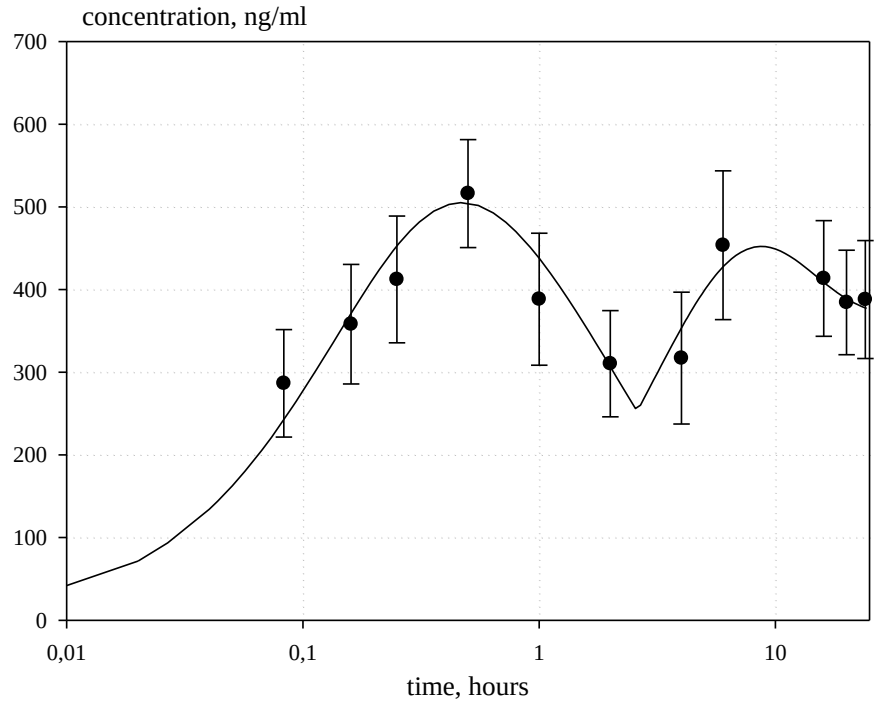

Fig.19

Time-based change of the Killevir preparation concentration in spleen following rectal administration in rats in dose 2.8 mg/kg

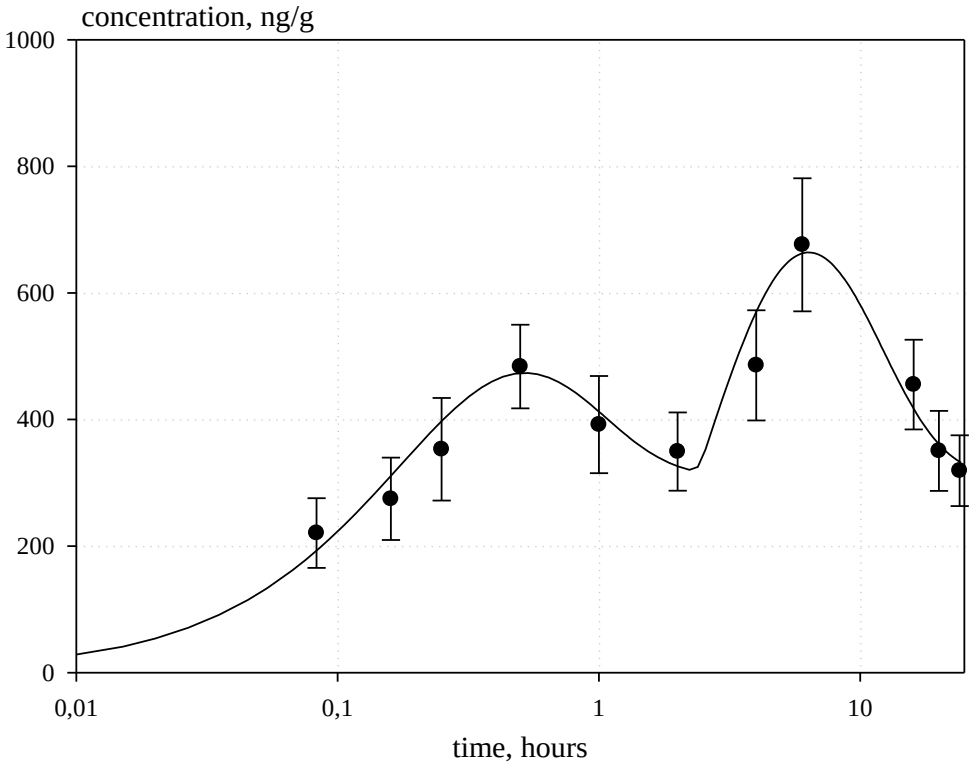

Fig.20

Time-based change of the Killevir preparation concentration in rats' epiploon following rectal administration in dose 2.8 mg/kg

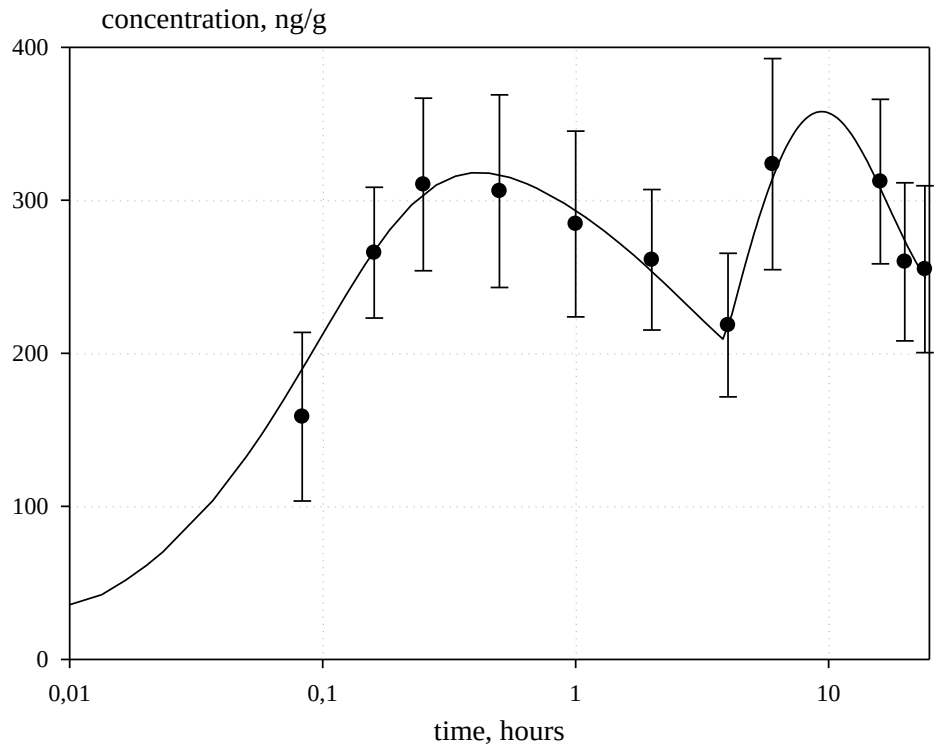

Fig.21

Time-based change of the Killevir preparation concentration in rats' kidney following rectal administration in dose 2.8 mg/kg

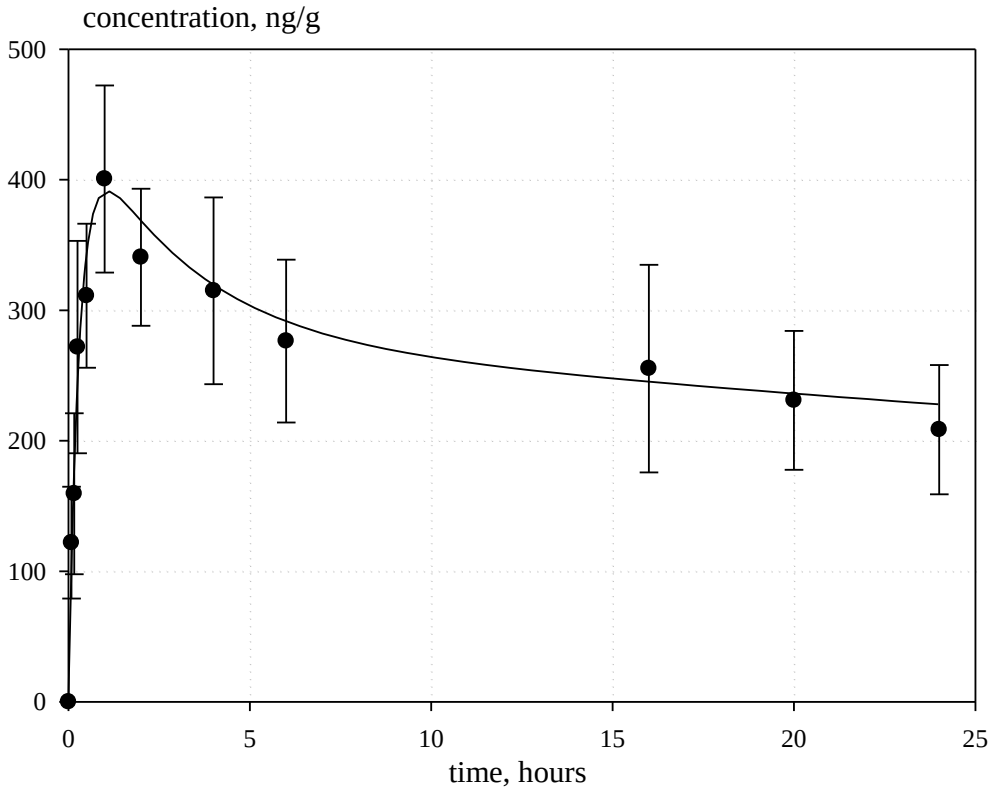

Fig.22

Time-based change of the Killevir preparation concentration in rats' liver following rectal administration in dose 2.8 mg/kg

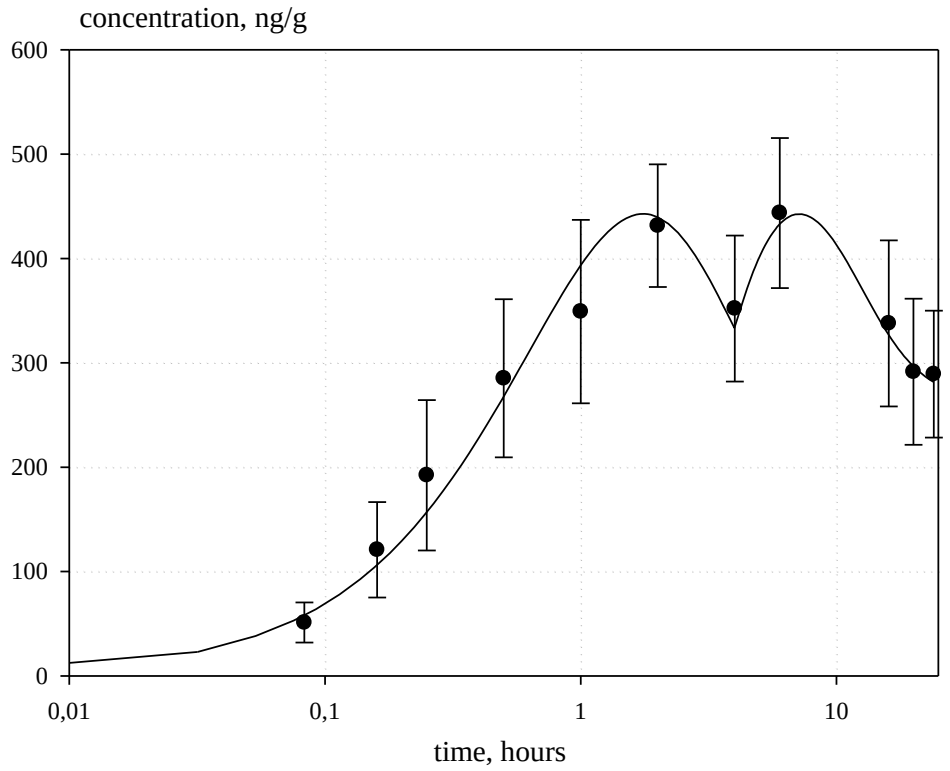

Fig.23

Time-based change of the Killevir preparation concentration in rats' muscles tissue following rectal administration in dose 2.8 mg/kg

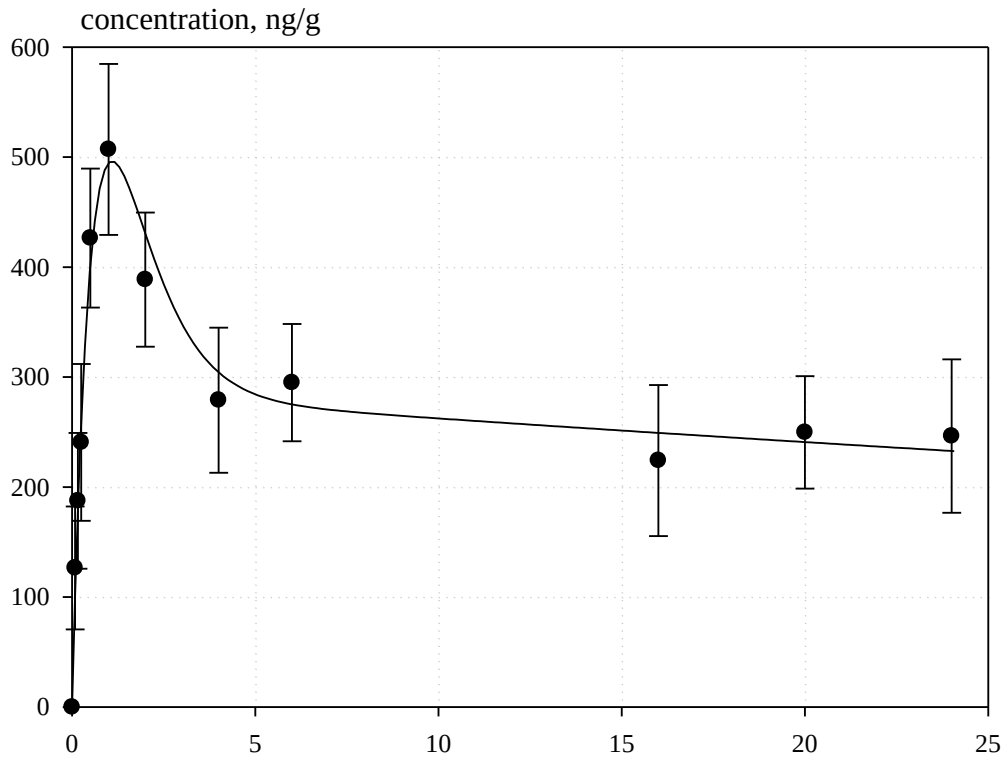

Fig 24

Time-based change of the Killevir preparation concentration in rats' brain following rectal administration in dose 2.8 mg/kg

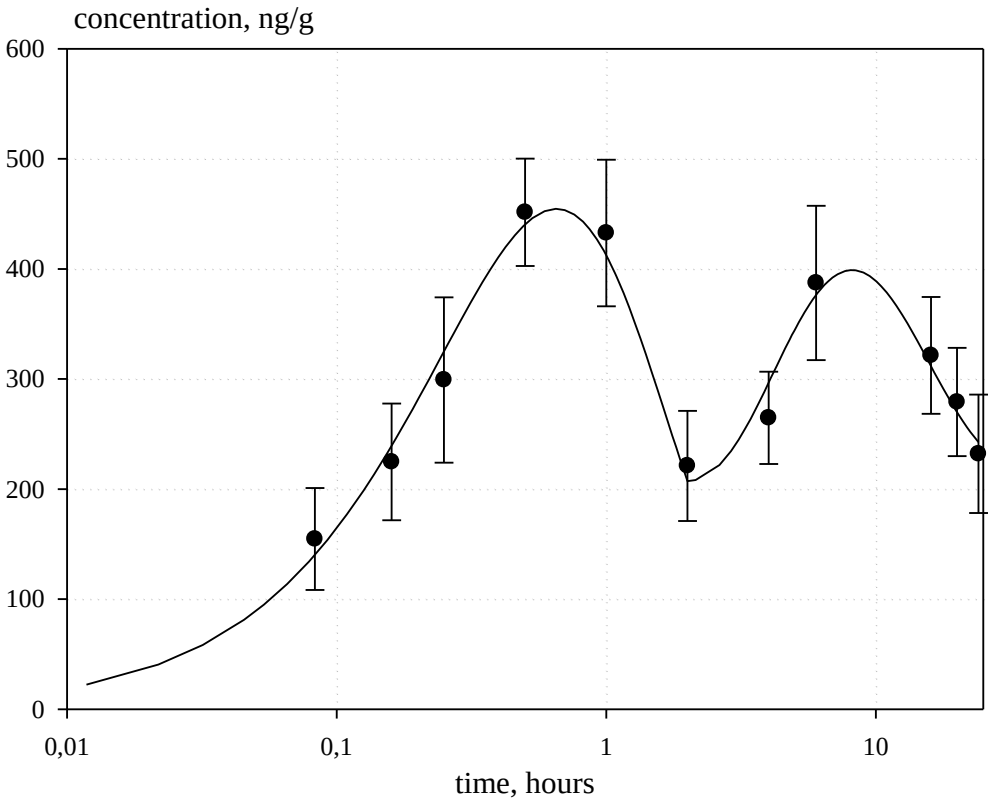

Fig.25

Time-based change of the Killevir preparation concentration in rats' lung tissue following rectal administration in rats in dose 2.8

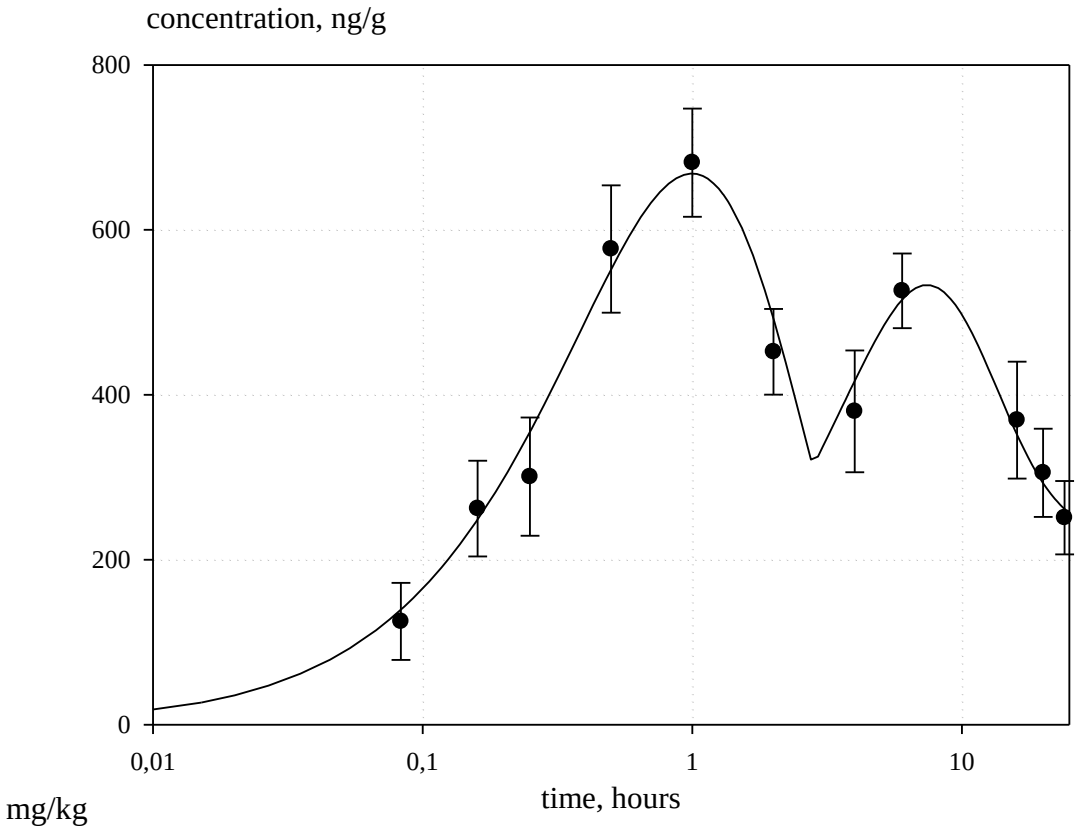

Fig.26

Таблица 29

Characteristics of the Killevir preparation distribution in rats' organs at rectal administration in dose 2.8 mg/kg

Test organ	AUC (ng/ml)*hour	AUMC (ng/ml)*hour^2	MRT hour	k_α/k_β .hour^{-1}	k_{ab} . hour^{-1}	Tmax. hour	Cmax. (1)/(2) ng/ml (g)
Blood	9800.1	247235.8	25.23	0.236	0.003	0.47/8.62	505.1/452.2
Lungs	9850.9	233580.4	23.71	0.241	0.325	0.99/7.50	668.2/533.1
Liver	8578.2	273030.7	31.83	0.011	0.032	1.76/7.32	443.8/442.4
Spleen	11406.4	431404.6	37.82	0.235	0.045	0.53/6.37	473.6/664.3
Kidney	6489.4	72061.8	11.10	0.330/ 0.009	3.288	1.08	391.3
Muscles	1090.0	8127.7	7.46	1.189/ 0.008	1.252	1.10	241.7
Epiploon	10570.3	340863.2	32.25	0.184	0.359	0.43/9.37	318.0/358.2
Brain	7698.9	194446.3	25.26	0.007	1.557	0.65/8.07	454.6/398.8

Note.

– *) k_β - for secondary curve;

– **) k_{ab} – for primary curve;

– values T_{max} and C_{max} are given for primary (1) and secondary (2) curves, respectively;

– the data on kidneys and muscles is given for unimodal profile.

2.1.4. Study of the preparation excretion in experiments on rats

The process of the Killevir preparation excretion with urine after intravenous and rectal administration was studied on rat groups (4 units in each group) at single i/v administration in doses 0.7 and 3.5 mg/kg and single rectal administration in dose 2.8 mg/kg. Results of evaluation of total amount of the preparation excreted with urine from animals are given in Tables 30-33, and the same data is presented graphically in Fig.27-30.

Table 30

Kinetics of the Killevir preparation excretion with urine following i/v administration in rats in dose 0.7 mg/kg (0.728 mg for four animals)

Time interval, hours	Preparation amount (μg) excreted during the time interval	Total preparation amount (μg) excreted during the time interval	Total preparation amount (% of the administered dose) excreted during the period starting with administration through the end of the corresponding time interval in the first column
0-1.5	3.23	3.23	0.44
1.5-3	22.59	25.82	3.55
3-6	18.54	44.36	6.09
6-24	19.09	63.45	8.72
24-28	4.44	67.89	9.32
28-48	6.00	73.89	10.15
48-72	3.86	77.75	10.68
72-96	2.48	80.23	11.02

Integral curve of the Killevir preparation excretion with urine following i/v administration in mice in dose 0.7 mg/kg

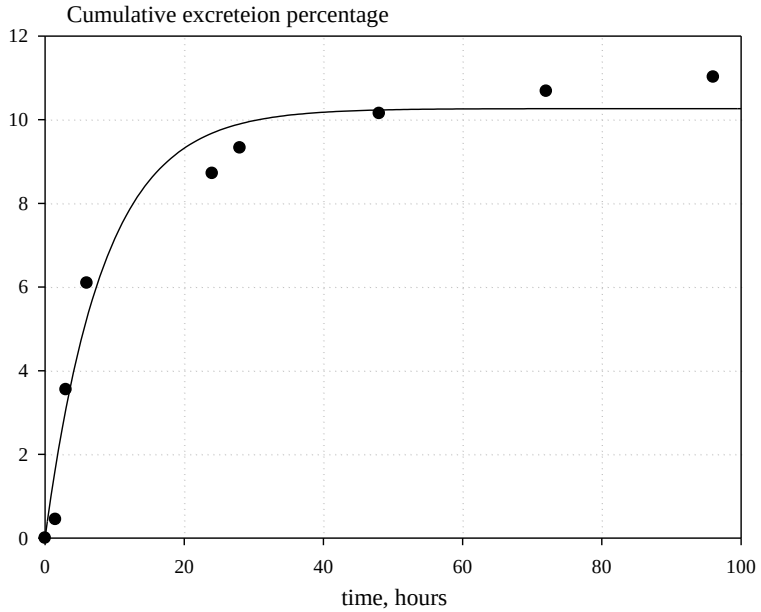

Fig.27.

Table 31

Kinetics of the Killevir preparation excretion with urine following i/v administration in rats in dose 3.5 mg/kg (4.20 mg for four animals)

Time interval, hours	Preparation amount (μg) excreted during the time interval	Total preparation amount (μg) excreted during the time interval	Total preparation amount (% of the administered dose) excreted during the period starting with administration through the end of the corresponding time interval in the first column
0-3	137.16	137.16	3.27
3-6	46.30	183.46	4.37
6-24	23.57	207.02	4.93
24-30	5.66	212.69	5.06
30-48	3.13	215.82	5.14
48-54	3.62	219.44	5.22
54-72	4.25	223.69	5.33
72-78	2.21	225.90	5.38
78-96	2.07	227.97	5.43

Integral curve of the Killevir preparation excretion with urine following i/v administration in mice in dose 3.5 mg/kg

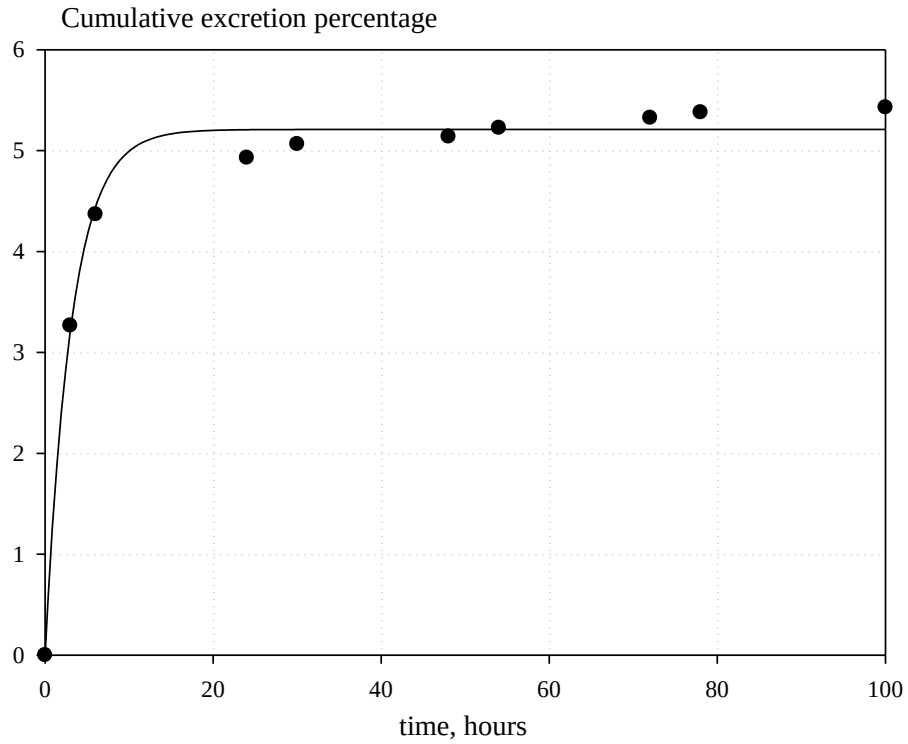

Fig.28

Table 32

Kinetics of the Killevir preparation excretion with urine following i/v administration in rats in dose 2.8 mg/kg (3.32 mg for four animals)

Time interval, hours	Preparation amount (μg) excreted during the time interval	Total preparation amount (μg) excreted during the time interval	Total preparation amount (% of the administered dose) excreted during the period starting with administration through the end of the corresponding time interval in the first column
0-3	51.13	51.13	1.54
3-6	21.58	72.71	2.19
6-24	12.96	85.67	2.58
24-30	5.64	91.31	2.75
30-48	5.31	96.62	2.91
48-54	6.31	102.93	3.10
54-72	1.33	104.26	3.14
72-76	2.65	106.91	3.22
76-96	1.99	108.90	3.28

Integral curve of the Killevir preparation excretion with urine following single rectal administration in mice in dose 2.8 mg/kg

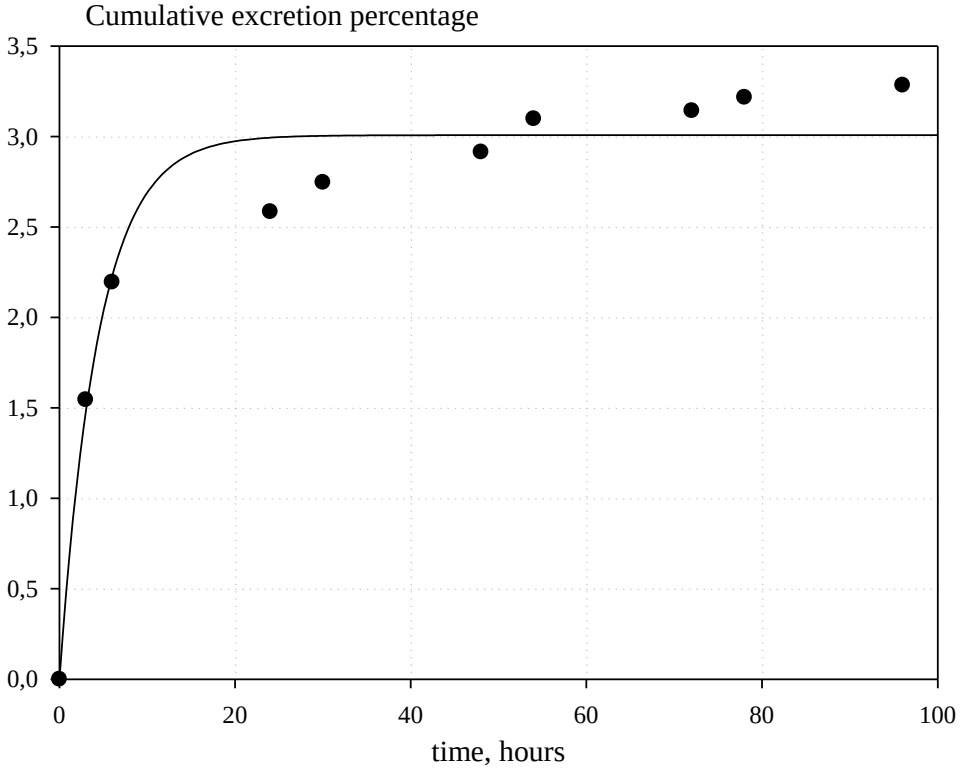

Fig.29

Analysis of the obtained data demonstrated that regardless the route of delivery the process of Killevir excretion in rats is satisfactory described by sigmoid equation:

$$M_t / D = a[1 - \exp(-bt)] \qquad (5)$$

where:

M_t/D - percent of amount of the preparation excreted during the time of observation t;

a (%), b($hour^{-1}$) – asymptomatic value of percent of the preparation amount, calculated within the time of observation and excretion constant, respectively.

At the same time, noteworthy is obvious circadian periodicity in behavior of cumulative curves conditioned not only by sampling periodicity.

Whereas for small accumulated percents of excretion M_t/D considering this effect is not essential, it is of value for significant indices of excretion. In this connection description of excretion was done by means of a more complicated ratio, which in some way considered circadian cycles:

$$M_t / D = \sum_i a_i (1 - \exp(-b_i (t - t_i))) \qquad (6)$$

Evidently, the ratio (6) is just the "expansion" of ratio (5) by introducing effective times t_i , the values of which correlated with circadian periodicity.

The results of excretion curves approximation are given in Table 34.

Table 34

Equitation (5) parameters at kidney excretion in rats

Preparation	Administration	Dose, mg/kg	a	b. $hour^{-1}$
Killevir	i/v	0.7	10.27	0.119
	i/v	3.5	5.21	0.319
	rectal	2.8	3.01	0.225

Since excretion is evidently non-stationary, there’s no sense in speaking of certain values of renal Cl_R=M/AUC and extrarenal Cl_{NR} = Cl-Cl_R clearances.

It follows from the given data that the Killevir preparation excretion with urine is very low and dose-dependent, which is conformed to the character of pharmacokinetic curves after intravenous administration given above.

2.2. Study of pharmacokinetics and excretion of the Killevir preparation in experiments on rabbits

Pharmacokinetics of the preparation was studied for 24 hours after single intravenous and rectal administration of the Killevir preparation in dose 3.5 μg/kg.

The results of the preparation concentration evaluation in rabbit blood after i/v administration of the preparation are given in Table 35, and the same data is presented graphically in Fig.30.

Table 35

Time-based change of the Killevir preparation concentration (ng/ml) in blood following i/v administration in rabbits in dose 3.50 mg /kg

Animal #	Preparation concentration (ng/ml) in different time intervals(hours)							
	0.083	0.25	0.5	1	2	4	6	24
1	7624.7	5048.8	1818.4	1078.6	664.8	616.5	395.2	224.3
2	7415.4	5255.58	1708.6	1012.8	723.2	536.0	457.7	266.8
average	7520.0	5152.2	1763.5	1045.7	694.0	576.3	426.4	245.5
St.deviation	148.0	146.1	77.7	46.5	41.2	56.9	44.3	30.1

Study of time-dependent concentration of the Killevir preparation in blood plasma after i/v administration in rabbits is described by bi-exponential model:

$$C_t = C_1 \exp(-k_\alpha t) + C_2 \exp(-k_\beta t) \qquad (7)$$

Time-based change of the Killevir preparation concentration (ng/ml) in blood following i/v administration in rabbits in dose 3.50 mg /kg

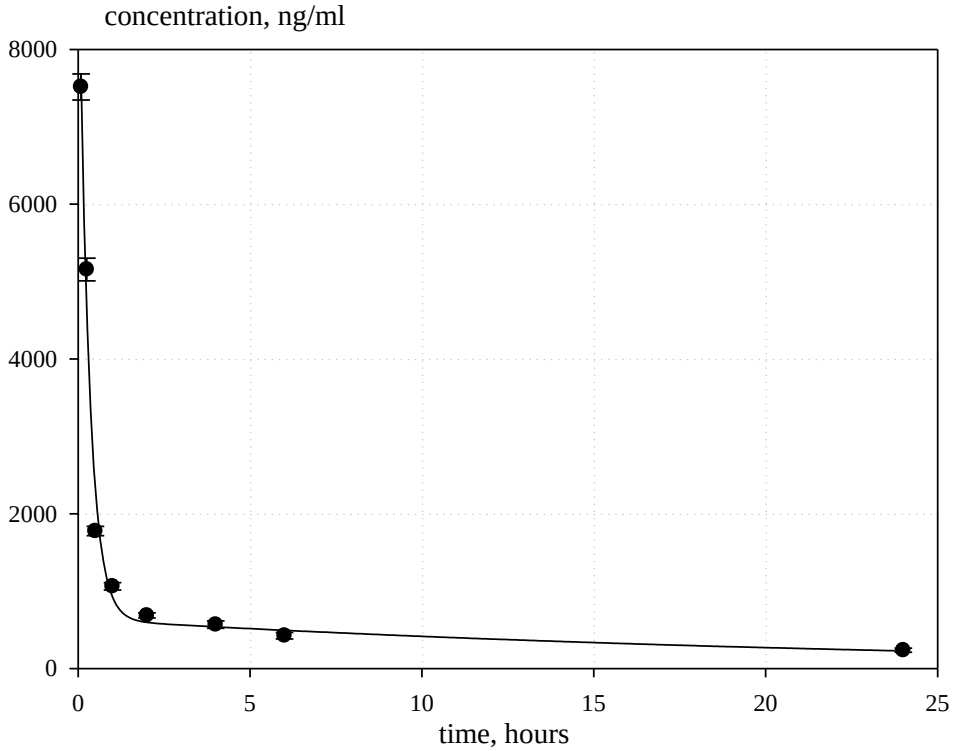

Fig.30

Based on the data from Table 35, the pharmacokinetic parameters of the Killevir preparation at i/v administration were calculated (Table 36).

Table 36

Pharmakokinetic parameters of the Killevir preparation in rabbits at i/v administration in dose 3.5 mg/kg

System parameters	Value
AUC. ng×hour/ml	12323.45
AUMC. ng×hour^2 /ml.	96284.82
MRT hour	7.81
CL. ml/hour	768
V ss. ml	6000.4
k_α./k_β . hour^{-1}	3.407/0.043

Killevir preparation behavior in blood after rectal administration is presented by the data given in Table 37 and Fig.31.

Table 37

Time-based change of the Killevir preparation concentration (ng/ml) in blood following rectal administration in rabbits in dose 3.50 mg /kg

Animal #	Preparation concentration (ng/ml)in different time intervals(hours)							
	0.083	0.25	0.5	1	2	4	6	24
1	330.3	373.9	410.4	446.1	331.9	391.0	361.9	246.2
2	262.7	299.4	335.0	546.8	405.3	304.8	291.7	185.7
average	296.5	336.7	372.7	496.5	368.6	347.9	326.8	216.0
St.deviation	47.8	52.7	53.3	71.2	51.9	61.0	49.7	42.8

Concentrations of the Killevir preparation in blood plasma after rectal administration in rabbits are described by “classic” equation (3).

Time-based change of the Killevir preparation concentration (ng/ml) in blood following rectal administration in rabbits in dose 3.50 mg /kg

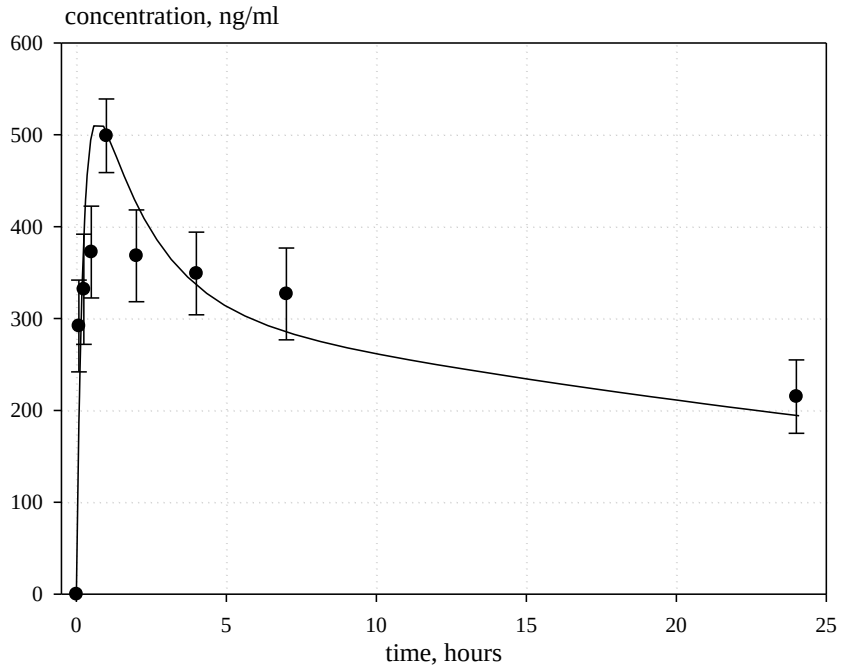

Fig.31

Table 38

Pharmacokinetic parameters of the Killevir preparation in rabbits at rectal administration in dose 3.5 mg/kg

System parameters	Value
AUC. ng×hour/ml	6534.0
AUMC. ng×hour^2 /ml.	67700.5
C_{max} .ng	515.2
T_{max}. hour	0.72
MRT. hour	10.36
CL. ml/hour	1410
V ss. ml	14607
k_α./k_β /k_{ab}. hour^{-1}	0.515/0.021/4.335

Study of renal excretion of the Killevir preparation in rabbits was conducted after single intravenous and rectal administration in dose 3.5 mg/kg.

The results of experiments are presented in Tables 39-40 and in Fig.32-33.

Table 39

Kinetics of the Killevir preparation excretion with urine following i/v administration in rabbits in dose 3.5 mg/kg

Time interval, hours	Preparation amount (μg) excreted during the time interval	Total preparation amount (μg) excreted during the time interval	Total preparation amount (% of the administered dose) excreted during the period starting with administration through the end of the corresponding time interval in the first column
0-3	760.5	760.5	8.05
3-6	42.0	802.5	8.49
6-24	160.2	962.7	10.19
24-28	4751.8	5714.4	60.47
28-48	230.6	5945.0	62.91
48-72	342.1	6287.1	66.53
72-120	647.3	6934.4	73.38

Kinetics of the Killevir preparation excretion with urine following i/v administration in rabbits in dose 3.5 mg/kg

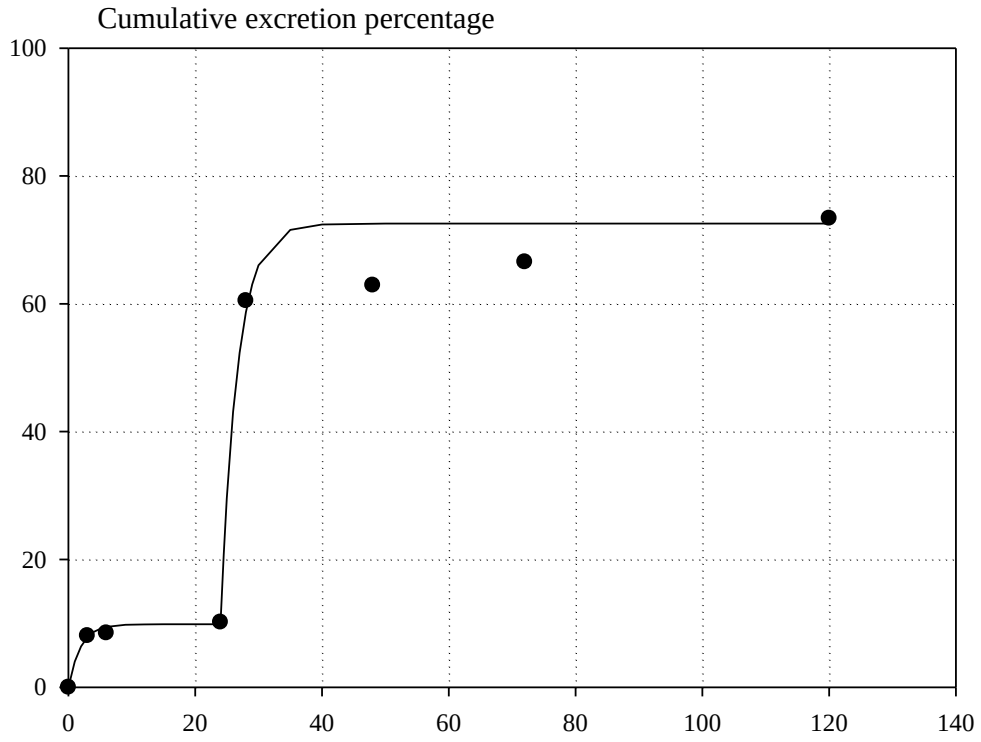

Fig.32

Table 40

Kinetics of the Killevir preparation excretion with urine following rectal administration in rabbits in dose 3.5 mg/kg

Time interval, hours	Preparation amount (μg) excreted during the time interval	Total preparation amount (μg) excreted during the time interval	Total preparation amount (% of the administered dose) excreted during the period starting with administration through the end of the corresponding time interval in the first column
0-2	386.1	386.1	4.17
2-7	340.7	726.8	7.85
7-24	399.1	1125.9	12.16
24-30	270.7	1396.6	15.08
30-48	368.5	1765.1	19.06
48-54	235.8	2000.9	21.61
54-72	259.5	2260.4	24.41
72-78	167.6	2428.0	26.22
78-90	337.7	2765.8	29.87

Kinetics of the Killevir preparation excretion with urine following rectal administration in rabbits in dose 3.5 mg/kg

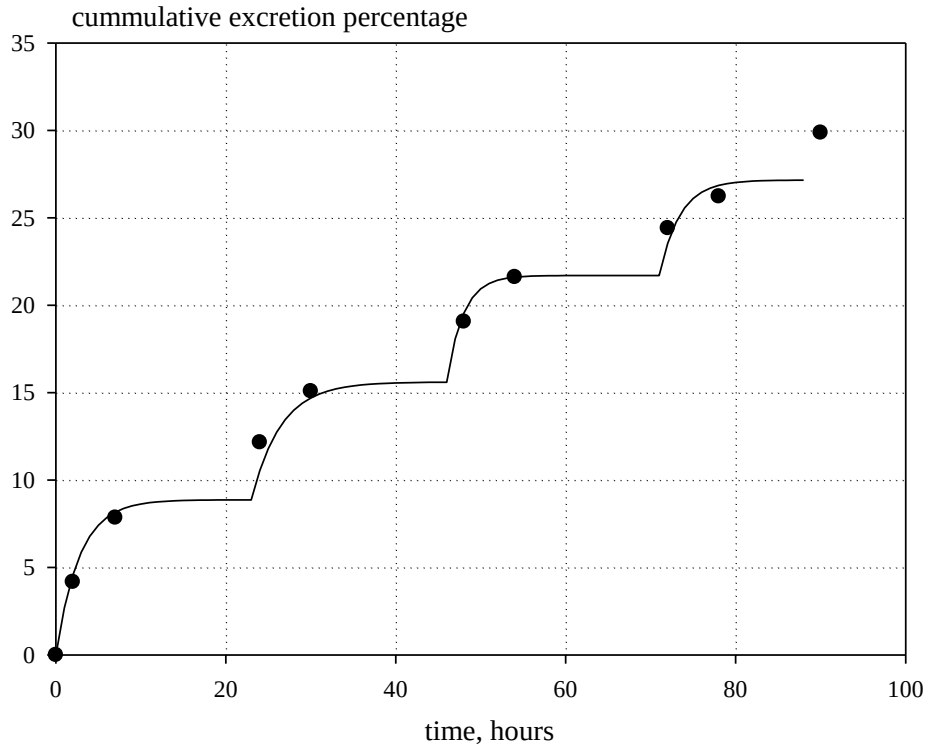

Fig.33

Table 41

Equitations (5) and (6) parameters at kidney Killevir preparation excretion in rabbits

Administration	Dose, mg/kg	a	b. hour^{-1}
i/v	3.5	9.86 62.71	0.524 3.721
rectal	3.5	8.87 6.73 6.09 5.46	0.363 0.286 0.521 0.138

Comparison of areas under pharmacokinetic curves at the same dosing (3.5 mg/kg) allows calculating relative interspecific bioavailability for rabbits and rats, that is as follows:

$f_м = AUC_{rabbits} / AUC_{mice} = 12323 / 11915 = 1.03$

CONCLUSION

Pharmacokinetics of the Killevir preparation therapeutic form was studied.

For assessment of pharmacokinetic parameters the appropriate mathematical models were chosen and pharmacokinetic parameters at intravenous and rectal administration were calculated.

Time–based changes of the preparation concentration in blood after intravenous and rectal administration in rats are described by different equations, at that their type is not "classic" relative to the proposed 2-part pharmacokinetic model with absorption.

Regardless the route of administration and animal species the Killevir preparation penetrates into the main organs and tissues.

Based on the results of experiments on rats at i/v administration (as the most reliable route with respect to the level of the registered count) a conclusion can be made on existing of dose-dependent relation between pharmacokinetic indices and the administered dose.

Relative interspecific bioavailability is 1.03, which evidences the absence of visible species-specificity (by "blood" index) in relation to the given preparation.

However, it should be mentioned that a range of indirect indices such as type of pharmacokinetic curve, cumulative percent of excretion, elimination constants – is the evidence of existing species-specificity. For example, excretion of the Killevir preparation at i/v administration is 5-11 % in rats (depending on dose) whereas the analogous index for rabbits is more than 70%. Similar dynamics is observed at rectal administration.

Bimodal type of pharmacokinetic curves is a very rare variant. Researchers present different interpretations of such result. For example, authors of the paper [7] make a conclusion, that bimodal manner of cefuroxime and ciprofloxacin concentration change provides for existence of two different transporting mechanisms in gastric mucosa. Study of two therapeutic forms of methylphenidate (capsules and tablets) [8], diminazene [9] and testosteron [10] has led to biphasic pharmacokinetics as well but without explanation variants of such type of curves. Atypical bimodality in blood was observed in case of studies of rofecoxibe metabolism [11] as well. Authors [12] explain the formation of bi- and even triphasic curves by non-linearity of relation coefficients of distribution and concentration (in the variant of Michaelis-Menten equation) and by ligands formation.

The actual mechanism of biphasic profiles formation within the frames of this study can only be guessed. Considering the fact that the studies were conducted with the labeled preparation and the "dips" in curves were detected within narrow time ranges, the preparation can be supposed to be deposited in organs, which were not included in the list of test ones (for example, adsorbed on surfaces of blood vessels).

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