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Report

Assessment of absolute bioavailability of Killevir dosage form at intramuscular administration

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ABBREVIATIONS

AUC - area under pharmacokinetic curve “concentration (C) - time (t)”;

AUMC - area under pharmacokinetic curve “concentration -time product (C x t)– time (t)”;

C_{max} - maximal concentration of substance (estimated value), ng/ml, ng/g;

T_{max} - time required to achieve C_{max} (estimated value), h;

V_{ss} - quasi-stationary volume of distribution, ml;

MRT – mean time of preparation residence in organism, min;

MRT_{im} - mean time of preparation residence in organism at i/m administration, h;

MRT_{iv} - mean time of preparation residence in organism at i/v administration, h;

f_{abs} – absolute bioavailability;

D - administered dose, ng;

Cl – total clearance, ml/min;

t – time, min, h;

ng – nanogram;

ml – milliliter;

i/v– intravenously;

i/m - intramuscularly

av. val. – average value;

ASD – average standard deviation at 95% significance level;

DMSO - dimethylsulfoxide

CONTENTS

Introduction

Key words: Killevir preparation, pharmacokinetics, intravenous administration, intramuscular administration, rabbits.

Report: 12 pages, 4 tables, 4 figures, 6 references

The study objective is assessment of Killevir dosage form distribution in blood with evaluation of the main pharacokinetic parameters at intravenous and intramuscular administration and assessment of Killevir absolute bioavailability.

In the studies Killevir solution in 50% DMSO was used.

1. Materials and methods

The studies were conducted at the Research Center for Toxicology and Hygienic Regulation of Biopreparations, FMBA, in accordance with "Guidelines on conducting preclinical pharmacokinetic studies of drugs" [1].

The experiments were carried out on Chinchilla male rabbits, body weight 2.0 – 2.5 kg.

Rabbits for the studies were purchased at "Manikhino" Animal Breeding Center.

The animals were kept in conventional cages under 12 h light/dark regime and had free access to water and feed.

Housing and handling animals met sanitary rules approved by the Ministry of Health of the USSR of 06.07.73, on construction, equipment and maintenance of experimental-biological clinics (vivariums). Animals received natural and peletted diet according to the standards approved by the Order of the Ministry of Health of the USSR #755 of 12.08.77. All animals were kept under quarantine and acclimated to laboratory conditions for 21 days prior to the start of dosing.

Quantitative determination of Killevir preparation concentration was performed by radioisotope method. For that, a part of hydrogen atoms in molecule of fulleren-polyaminocapronic acid were preliminarily substituted by ^{3}H (tritium) atoms by method of three-phase catalysis. As a result, for testing the customer supplied the preparation with the following characteristics - (^{3}H) Killevir solution in DMSO with bulk activity 1 mCi/ml and specific activity 0.4 mCi/mg.

Weights of animals were determined before dosing on electronic balance PV-15.

Mass of biosamples was determined on laboratory balance Explorer Pro EP214C (Ohaus, Switzerland).

Radioactivity of whole blood samples 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 4 g/l and 0.1 g/l, respectively, and 30% v/v triton-X100.

2.1. Determination of Killevir in biological samples

Distribution of the preparation in blood of rabbits was studied during 24 hours after a single i/v (in auricular vein) and i/m (in caudal femoral muscule) administration of (^{3}H) Killevir in 50% DMSO in dose 1.4 mg/kg. At both routes in definite time intervals after injections the cuts were done on marginal auricular vein and blood samples of about 0.2 ml were taken. Control time points at i/v route - 5, 15, 30, 60, 120, 240, 360, and 1440 min, at i/m route - 10, 20, 30, 60, 120, 240, 360, and 1440 min after injection.

In both cases the volume of the preparation to be administered was correlated with mass of each laboratory animal.

2.2. Preparation of samples

In glass volumetric flasks of 10cm^3 a sample of whole blood approximately 200 mg was placed and fixed; in the flask 0.6 ml of $HClO_4$(c.) + HNO_3(c.) acid mixture (1:1) were added and the flask was sealed with Parafilm. The prepared flasks were placed above bain-marie, water temperature $\approx 70^0$C, and kept for 2 hours until the mixture is cleared. Then the flasks were cooled and the volume was brought to 1 ml with above-indicated acid mixture.

From the obtained samples 25 μl aliquots were taken with variable-volume automatic pipette and added to 10 ml of scintillator in scintillating flask; the flask was shaken up and placed for 2-3 hours in dark place at room temperature.

After that the samples are prepared for taking measurements on scintillation counter.

2.3. Calibration

To shift from the measured radioactivity to concentration of the preparation in blood, a calibration diagram was built describing the level of counting of radioactivly labelled Killevir preparation as a part of control animals blood mineralisate depending on its concentration in standard scintillator volume (Table 1, Fig.1). The calibration diagram calculated by regression method in range 0.6-60 ng/g is described by linear equation:

$$y=430.5 + 1783*x \qquad (1)$$

where y – number of scintillation acts per minute;

x – concentration of Killevir preparation, ng/g.

Concentration of the preparation in test sample was determined using ratio (1) of the diagram based on measurements of radioactivity and mass of the test samples.

Table 1

Dependence of radioactivity of averaged by organs calibration samples on concentration of labeled Killevir preparation

Concentration. ng/ml	Radioactivity, (number of impulses per minute)	
	Average	Standard deviation
0.6000	1154.2	372.4
6.0000	11191.2	556.3
15.0000	27351.0	752.0
30.0000	55139.0	1120.0
60.0000	106755.6	2496.9

Dependence of samples radioactivity on concentration of labeled Killevir preparation

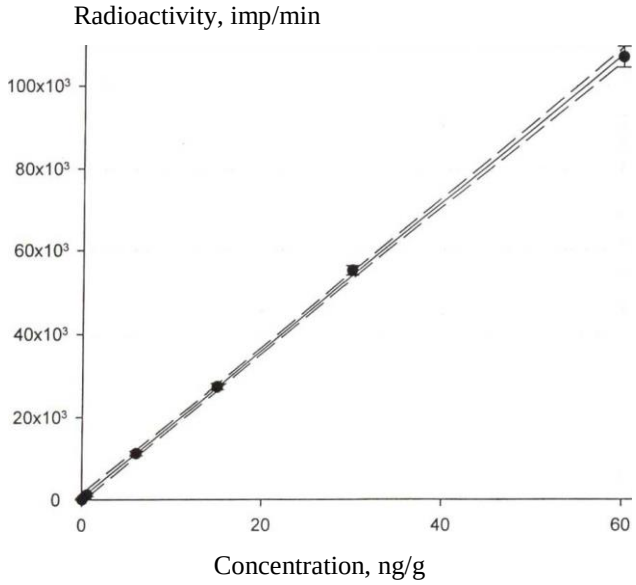


Fig.1.

2.4. Analysis of pharmacokinetic data

Estimation of integral parameters of the preparation pharmacokinetics was performed using 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 estimated values of AUC and AUMC areas under pharmacokintetic curves at definite dose D of the preparation the following systemic parameters were determined:

$$MRT = AUMC/AUC$$

$$Cl = D/AUC$$

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

$$f_{abs} = AUC_{sub}/AUC_{iv}$$

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

For i/m administration C_{max} and T_{max} were calculated.

3. Study of Killevir preparation pharmacokinetics in experiments on rabbits

Background values of activity were obtained from study of control animals' blood samples and taken into account when building calibration line (see 2.3).

Values of the preparation concentrations in organs and tissues examined after different time intervals following i/v and i/m injections of the preparation in dose 1.4 mg/kg are given in Tables 2-3; in Fig. 2-3 they are presented graphically.

Table 2

Time-dependent change of Killevir concentration (ng/ml) in blood following i/v administration in rabbits in dose 1.4 mg /kg

Animal #	Concentration (ng/ml) in different time intervals(min)							
	5	15	30	60	120	240	360	1440
1	300.5	187.1	84.2	18.5	17.7	22.6	21.0	0.7
2	350.7	137.8	54.5	43.1	16.6	10.3	3.6	2.5
3	267.2	152.5	48.8	28.1	35.5	15.9	20.2	0.9
av. val.	306.1	159.1	62.5	29.9	23.3	16.3	14.9	1.4
SD	42.1	25.3	19.0	12.4	10.6	6.2	9.8	1.0

Table 3

Time-dependent change of Killevir concentration (ng/ml) in blood following i/m administration in rabbits in dose 1.4 mg /kg

Animal #	Concentration (ng/ml) in different time intervals(min)							
	10	20	30	60	120	240	360	1440
1	7.6	8.9	4.6	4.8	16.9	9.0	6.3	U
2	4.1	6.9	4.0	3.9	10.7	16.2	8.0	1.7
3	5.0	9.8	5.8	7.4	11.3	9.6	9.1	0.7
av. val.	5.6	8.5	4.8	5.4	13.0	11.6	7.8	1.1
SD	1.8	1.5	0.9	1.8	3.4	4.0	1.4	0.5

Analysis of the data obtained demonstrated that time-dependent change of Killevir preparation concentration in blood after i/v administration in rabbits is quite satisfactorily described by biexponential curve [5]:

$$C_t = C_1 \exp(- k_\alpha t) + C_2 \exp(- k_\beta t) \qquad (2)$$

and after i/m administration – by bimodal curve which was used in [6]:

$$C_t = \sum_{i=1.3} C_i \text{ x } \exp(- k_i t) + \sum_{i=4.6} C_i \text{ x } \exp[- k_i (t-t_0)] \qquad (3)$$

Time-dependent change of the Killevir concentration in blood following i/v administration in rabbits in dose 1.4 mg /kg

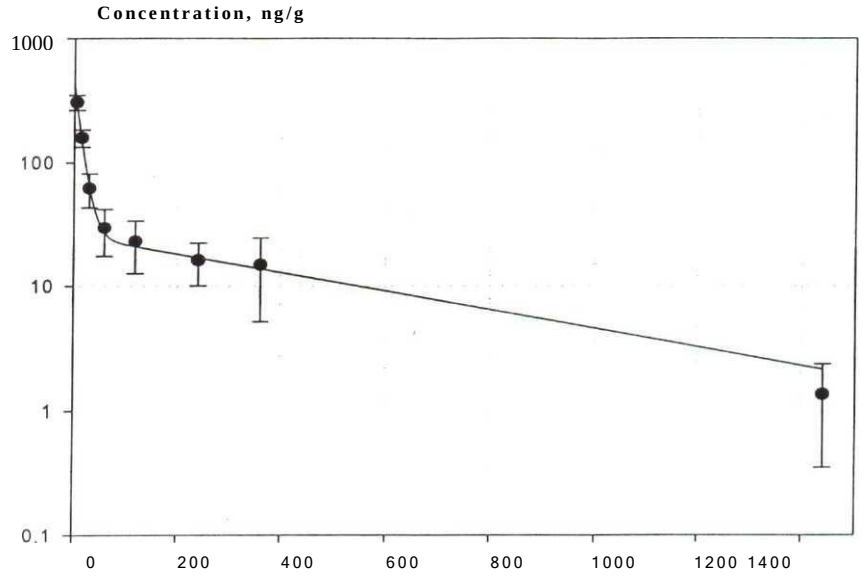
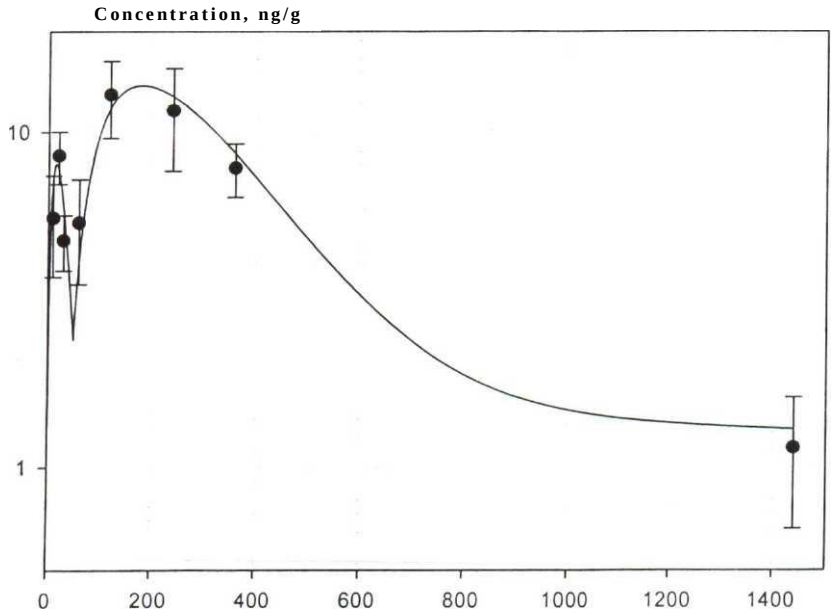


Fig.3

Time-dependent change of the Killevir concentration in blood following i/m administration in rabbits in dose 1.4 mg /kg



Time, min

Fig. 4

When assessing systemic parameters considering blood density in healthy animals (1050 kg/m^3 at t=20°C) the obtained concentrations of the preparation in ng/g were identical to those expressed in ng/ml. The main parameters are presented in Table 4.

Table 4

Pharmacokinetic parameters of Killevir in rabbits at i/v and i/m injections in dose 1.4 mg/kg

Route	AUC, ng×min/ml	AUMC, ng×min/ml	MRT, min	CI, ml/min	V_{ss}, l	k_a,/ k_β/k_{abs}, min^{-1}
i/v	18846.6	6.188*10^6	328	74.3	24.4	0.0774/0.00173
i/m	5963.2	2.691*10^6	451	234.8	105.9	0.0528/0.0533/0.0602 0.00824/0.00774 /0.000095

Time for achieving maximal T_{max} at the given parameters is 15 min and 180 min at concentrations C_{max} = 8.0 and 14.0 ng/ml, respectively.

Absolute bioavailability at i/m injection was f_{abs} = 31.6%.

CONCLUSION

Pharmacokinetics of Killevir dosage form was studied. Time-dependent changes of the preparation concentrations in blood of rabbits following intravenous and intramuscular administration are described by different equations: for intravenous administration – by standard biexponential variant, for intramuscular – by bimodal variant already used in the studies of Killevir pharmacokinetics.

Based on results obtained Killevir can be described as preparation with a long-time persistance in blood of the given animal species which is confirmed by the time of detection and stationary volumes of distribution.

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