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Report

"Detection of the Killevir preparation medicinal form absolute bioaccessibility at an intramuscular infusion"
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LIST OF ABBREVIATIONS

AVC – an area under a pharmacokinetic curve "concentration (c) – time (t)";
AUMC – an are under a pharmacokinetic curve "production of time to the preparation concentration (c x t) – time (t)";
C_{max} – a maximum concentration of a substance (a rated value), ng/ml, ng/g;
T_{max} – a time of the C_{max} achievement (a rated value), hour;
V_{ss} – a quasi-stationary volume of distribution, ml;
MRT – an average time of the preparation staying in the organism, min;
MRT_{intra} - an average time of the preparation staying in the organism in hours at an intramuscular infusion, hour;
MRT_{intra} - an average time of the preparation staying in the organism in hours at an intravenous infusion, hour;
fabs – absolute bioaccessibility;
D – infused dose, ng;
V_{ss} – a quasi-stationary volume of distribution, ml;
Cl – a general clearance, ml/min;
t – time, min. hour;
ng – nanogram;
ml – milliliter;
i/v – intravenously;
i/m – intramusculary;
aver. mean. – average meaning of a value;
st. dev. – standard deviation at a significance level 95%;
DMSO – dimethylsulfoxide

Contents

Introduction

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

Report: pages – 12, tables 4, figures 4, references – 6 names.

The aim of investigations consisted in a study of the "Killevir" preparation medicinal form distribution in blood with assessment of the basic pharmacokinetic parameters at the intravenous and intramuscular infusions, as well as in assessment of their absolute bioaccessibility. A solution of the "Killevir" preparation in an aqueous 50% solution of a DMSO was a medicinal form.

Materials and methods

The tests were conducted on a basis of the SRC of toxicology and hygienic reglamentation of the biopreparations of the RF Ministry of Public Health (MPH) according

to the "Methodical recommendations on a preclinical study of the medicinal preparation pharmacokinetics [1].

The rabbits-males of the "Shinshilla" breed with a body mass of 2,0-2,5 kg were used in experiments.

The rabbits of the shinshilla breed were acquired in a nursery of the laboratory animals "Manikhino".

The animals were kept in the standard cages in conditions of a 12-hour mode of lighting and a free access to feed and water.

A maintenance of animals corresponded to the sanitary rules, approved by the USSR MPH on 73.07.06, on arranging, equipping and maintenance of the experimentally-biological clinics (vivaria). The animals were fed by natural and briquette fodders according to the standards approved by the USSR MPH order № 755 of 77.08.12. Before the experiments the animals have been passing a quarantine and acclimatization in conditions of vivarium for 21 days.

A quantitative detection of the Killevir preparation concentration was made by a radioisotopic method. For that purpose, a part of the hydrogen atoms in a molecule of a fullerenepolyaminocaproic acid was preliminary substituted for the ^{3}H atoms (a tritium) by the method of a solid-phase catalysis. As a result of a substitution at the tests, the Customer has presented a preparation with the following characteristics: the Killevir solution (^{3}H) in a dimethylsulfoxide (DMSO) with a specific activity of 0,4 mKi/mg.

The animal mass was detected at the electronic balance "PV-15" before the preparation infusion.

The Biotest mass was detected at the laboratory balance "Explorer Pro EP214C"(the Ohaus firm, Switzerland).

A radioactivity of the undiluted blood tests, taken from the animals, was measured on a liquid scintillated counter "Triathler 425-034" of the Hidex firm, Finland [2].

A scintillator consisted of the PPO (2,5-phenyloxazol) and POPOP (1,4 – bis 5 – phenyloxazolyl benzene) solution in a toluol, 4 g/l and 0,1 g/l, respectively. Besides, the scintillator contained 30% v/v of the triton – x100.

2.1 Detection of the Killevir preparation in the biological samples

A distribution of the preparation in blood of the rabbits has been studied during 24 hours after a single infusion of the Killevir (^{3}H) into a DMSO solution 50% intravenously and intramusculary in a dose of 1,4 mg/kg.

The preparation was infused to the rabbits intravenously (to the otic vein) and intramusculary to the caudal femoral muscle. In both cases an incision on a marginal otic vein was made in the certain intervals of time after infusion and approximately 0,2 ml of blood was taken. The control temporary points for the intravenous infusion were – 5, 15, 30, 60, 120, 240, 360 and 1440 min., and for the intramuscular one – 10, 20, 30, 60, 120, 240, 360 and 1440 min. after a moment of infusion

In all the cases, an amount of the infised preparation was correlated with the certain laboratory animal weight.

2.2 Preparation of tests.

About 200 mg of the undiluted blood sample were placed to a glass measured test-tube with a 10 cm^3 volume and the weight was fixed; 0,6 ml of the $HClO_4$(c.)+HNO_3(c.) acid mixture in a ratio of 1:1 was added to a test-tube and plugged with a film "Parafilm". Thus prepared test-tubes were established in a test-tube rack on a water bath with a water temperature of≈70°C and have been sustained till a full clarity of the test-tube contents for 2 hours. A volume of the tests was brought to 1 ml of the initial acid mixture after cooling.

A 25 mkl aliquota was selected with an automatic pipette of a variable volume from the thus received samples and added to 10 ml of a scintillator in a scintillated flesk, the flask was shaked and left for 2-3 hours at a room temperature in a dark place.

After that, the tests were ready for measurements on a scintillated counter.

2.3 Calibration

A calibrating graph was made for transition from the measured radioactivity to the preparation concentration in blood – a level of the "Killevir" radioactively labeled preparation count in a blood mineralizate of the control animals, depending on its concentration in a standard volume of a scintellator (table 1, fig. 1). A calibrating graph calculated by a method of regression in a range of 0,6-60 ng/g is described by a linear equation:

$y=430,5+1783 \cdot x$ (1)

where: y is a number of the scintillation acts in a minute; x is a concentration of the Killevir preparation, ng/g;

The preparation concentration in an experimental test was detected with a use of the graph (1) ratio according to the measurements of radioactivity and the investigating test mass.

Table 1

Dependence of the calibrating test, averaged on the organs, radioactivity on a concentration of the Killevir Labeled preparation

Concentration, ng/ml	Radioactivity, (number of impulses/min.)	
	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

Fig. 1. Dependence of the test radioactivity on a concentration of the Killevir "labeled" preparation

1. Radioactivity, imp./min.
2. Concentration, ng/g

2.4 Analysis of the pharmacokinetic data.

A calculating of the preparation pharmacokinetics integral parameters was made according to the experimentally detected temporary row of concentrations c=c(t) in a blood plasma. According to recommendations [1, 4, 5], a calculation included a calculation of the basic pharmacokinetic parameters: AUC and AUMC.

The following systemic parameters were detected according to the calculated meanings of the AUC and AUMC areas under the pharmacokinetic curves at a certain dose D of the infused preparation:

MRT=AUMC/AUC

Cl=D/AUC

V_{ss}=ClxMRT

fabs=AUC_{sub}/AUC_{iv}

The program packages "SigmaPlot" and "Table Curve 2D" were used for analysis of experimental data (selection of the approximation models) and a statistical analysis was made in the frames of the "Exel" program. A calculation of the AUC and AUMC was made with a use of the Advanced Grefter program or by means of a numerical integration (Exel).

The C_{max} and T_{max} were detected for the intramuscular method of infusion.

3. Study of the Killevir preparation pharmacokinetics in the experiments on rabbits.

The background meanings of activity were received from the blood test investigation in animals from the control groups and considered in a procedure of a calibrating straight line construction (see p. 2.3).

The meanings of the preparation concentrations in the organs and tissues, investigated through the different intervals of time after the preparation i/v and i/m infusions in the 1,4 mg/kg doses are presented in the tables 2-3, the same data is presented in figs. 2-3 graphically.

Table 2.

Alteration of the Killevir preparation concentration (ng/g) from a time in blood after the preparation intravenous infusion to rabbits in a dose of 1,4 mg/kg

Animal number	The preparation concentration (ng/g) through the different intervals of time, (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
average meaning	306.1	159.1	62.5	29.9	23.3	16.3	14.9	1.4
standard deviation	42.1	25.3	19.0	12.4	10.6	6.2	9.8	1.0

Table 3

Alteration of the Killevir preparation concentration (ng/g) from a time in blood after the preparation intramuscular infusion to rabbits in a dose of 1,4 mg/kg

Animal number	The preparation concentration (ng/g) through the different intervals of time, (min.)							
	10	20	30	60	120	240	360	1440
1	7.6	8.9	4.6	4.8	16.9	9.0	6.3	1.1
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
average meaning	5.6	8.5	4.8	5.4	13.0	11.6	7.8	1.1
standard deviation	1.8	1.5	0.9	1.8	3.4	4.0	1.4	0.5

An analysis of the received data has demonstrated that an alteration in time of the Killevir preparation concentration in blood after its i/v infusion to rabbits is sufficiently satisfactorily described by a biexponential curve [5].

$$C_1=C_1\exp(-k_at)+C_2\exp(-k_Bt) \qquad (2)$$

and after a bimodal curve i/m infusion, which was used in [6]:

$$C_i=\sum C_i \times \exp(-k_i t)+ \sum C_i \times \exp[-k_i(t-t_0)] \quad (3)$$

Fig. 3. Alteration in time of the Killevir preparation concentration in blood of the rabbits after the intravenous infusion in a dose of 1,4 mg/kg

1. Concentration, ng/g
2. Time, min

Fig.4. Alteration in time of the Killevir preparation concentration in blood of the rabbits after the intramuscular infusion in a dose of 1,4 mg/kg

1. Concentration, ng/g
2. Time, min

At an assessment of the systemic parameters considering the blood density in healthy animals (1050 kg/m^3 at t=20°C), the received concentrations of the preparation ng/g were equal to concentrations expressed in ng/ml. The basic parameters are presented in a table 4.

Table 4.

Method of infusion	AUC NgxMin/ml	AUMC NgxMin^2/ml	MRT min	Cl Ml/min	V_{ss}, L	K_α,/K_β,/K_{abs}, min^{-1}
i/v	18846.6	$6.188*10^5$	328	74.3	24.4	0.0774/0.00173
i/m	5963.2	$2.691*10^5$	451	234.8	105.9	0.0528/0.0533/ 0.0602/0.00824/ 0.00774/0.000095

Pharmacokinetic parameters of the Killevir preparation in rabbits at the intravenous and intramuscular infusions in doses of 1,4 mg/kg

A time of the T_{max} maxima achievement at the given parameters of the curves is 15 and 180 min. with the C_{max} concentrations = 8,0 and 14,0 ^{2}/ml, respectively.

An absolute bioaccessibility for the intramuscular infusion was f_{abs}=31,6%.

Conclusion

Apharmacokinetics of the Killevir preparation medicinal form is studied.

The alterations in time of the preparation concentration in blood after its intravenous and intramuscular infusion to rabbits are described by the different levels:

- a classical bioexponential variant is used for the intravenous infusion;
- the earlier encountered in investigation of the Killevir pharmacokinetics bimodal variant is used for the intramuscular infusion.

According to the results of the conducted investigations, the preparation can be characterized as one possessing of a durative persistence in blood of experimental animals of the given type. That fact is supported by the times of detection and the stationary volumes of distribution.

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