DOI 10.1007/s11094-021-02454-5
Pharmaceutical Chemistry Journal, Vol. 55, No. 6, September, 2021 (Russian Original Vol. 55, No. 6, June, 2021)

MOLECULAR-BIOLOGICAL PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

PHARMACOKINETIC PARAMETERS OF THE LAPPACONITINE, GLYCYRRHIZIC ACID AND METHYLURACIL COMBINATION EXHIBITING ANTIARRHYTHMIC PROPERTIES UPON SINGLE INTRAGASTRIC ADMINISTRATION IN VARIOUS DOSES

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 55, No. 6, pp. 3 – 7, June, 2021.

Original article submitted October 20, 2020.

The pharmacokinetic properties of a finished dosage form (FDF) of a combination of lappaconitine, glycyrrhizic acid, and methyluracil (LGM) possessing antiarrhythmic activity were studied. The FDF of LGM upon a single intragastric administration in various doses [1680, 3360, and 6720 mg/kg in terms of lappaconitine (LC)] was rapidly absorbed from the gastrointestinal tract with the maximum blood-plasma concentration of LC being reached 15 min after drug intake. LC had a short elimination half-life and retention time in the body. The drug was extensively distributed throughout organs and tissues and exhibited tropism for the kidneys, liver, and myocardium. LC was metabolized to form *N*-deacetyllappaconitine (*N*-DLC). Its metabolite was detected in urine for at least 72 h, which indicated predominant excretion of the drug through the kidneys. The pharmacokinetic profile of *N*-DLC was similar to that of LC.

Keywords: lappaconitine, pharmacokinetic parameters, active metabolite *N*-deacetyllappaconitine.

Therapy with antiarrhythmic drugs is associated with a significant risk of manifesting arrhythmogenic effects, which leads to degraded patient condition and (or) the development of a previously unobserved type of arrhythmia [1]. Expansion of the arsenal of antiarrhythmics by discovering and developing new highly efficacious and safe drugs is considered the main pathway to optimization of arrhythmia pharmacotherapy. Preclinical studies of a combination of lappaconitine, glycyrrhizic acid, and methyluracil in several arrhythmia models found that it possessed potent antiarrhythmic activity and low toxicity and might be promising as a basis for the development of a new drug for treating arrhythmia [2, 3].

Determination of the pharmacokinetic parameters for adsorption, distribution, metabolism, and excretion is an important stage of new drug development. The administration pathways *in vivo* and the tissues in which drugs accumulate most and/or are retained longest are defined by knowing their pharmacokinetic properties. Pharmacokinetic studies of new drugs are necessary to find the concentration–effect relationship, which can be used to predict their activity in man, to establish the drug concentration in blood (plasma) or its rate of decrease, and to select an approximate dosing regimen [4, 5].

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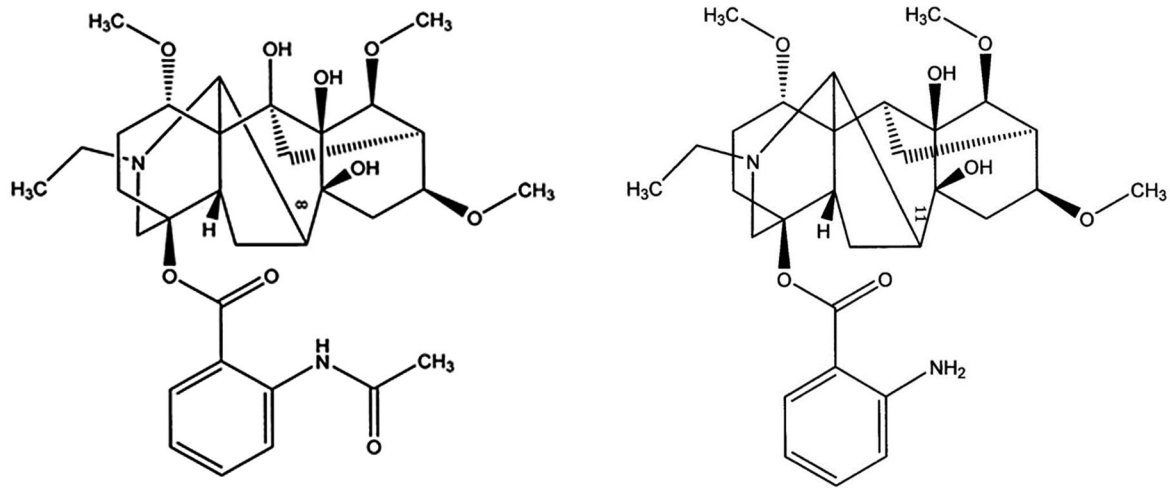

Lappaconitine *N*-Deacetyllappaconitine

Fig. 1. Structural formulas of lappaconitine and *N*-deacetyllappaconitine.

Therefore, the aim of the present research was to study the pharmacokinetics of a finished dosage form (FDF) based on a combination of lappaconitine (LC), glycyrrhizic acid, and methyluracil (LGM) that manifested antiarrhythmic properties in several arrhythmia models.

EXPERIMENTAL PART

The experiments used male inbred white rats (210 – 230 g) that were kept and cared for according to recommendations of the Russian Federation national standard GOST R-33044-2014 (Good Laboratory Practice Rules) and the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (The European Convention, 1986). The studies were conducted in compliance with requirements of MH RF Order No. 199n of Apr. 1, 2016 "On Approval of Good Laboratory Practice Rules" and Directives 2010/63/EU of the European Parliament and of the European Union Council of Sept. 22, 2010, on the protection of animals used for scientific purposes [6].

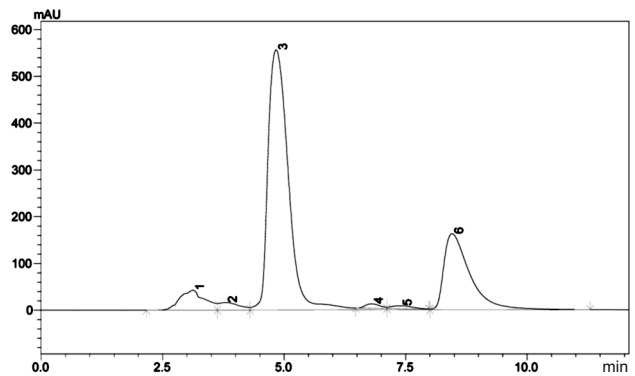

Fig. 2. Chromatogram of a mixture of reference standards of LC (0.05 mg/mL, t_R = 4.8 min) and *N*-DLC (0.05 mg/mL, t_R = 8.5 min).

Animals were stratified by body mass (body-mass deviations within groups were ≤10%) and were deprived of feed 12 h before the start of the experiment Working dilutions of aqueous solutions of the FDF of LGM were prepared *ex tempore* and were injected into the rats once intragastrically in doses of 1680, 3360, and 6720 μg/kg (recalculated for LC). Animals in the negative control group received distilled water (placebo). Each group included five animals.

Blood, kidneys, heart, and liver were harvested (after decapitation of the rats) 5, 15, 30, 60, 120, 240, 360, and 1440 min after intragastric injection. Urine was collected in the intervals 0 – 3, 3 – 6, 6 – 12, 12 – 18, 18 – 24, 24 – 48, and 48 – 72 h.

The studies used a Shimadzu (Japan) LC-20 Prominence liquid chromatograph with an SPD-20A diode-array detector. LC and its metabolite *N*-deacetyllappaconitine (*N*-DLC), which was prepared in laboratories of Ufa Inst. Chem., Russ. Acad. Sci. (UfIC, RAS), were determined at 254 nm using a Luna NH_2 column (250 × 4.6 mm, 5 μm; Phenomenex). The mobile phase was a mixture of hexane and *i*-PrOH (75:25).

The pharmacokinetic properties of the studied drugs were assessed by calculating parameters such as the area under the pharmacokinetic concentration—time curve (*AUC*) by a model-independent method of statistical moments [7]; total (apparent) clearance (*Cl*); elimination constant (K_{el}); total (apparent) distribution volume (V_d); elimination half-life ($T_{1/2}$); and average retention time characterizing the mean residence time *in vivo* of the drug (*MRT*) (h). The degree of accumulation of the compounds in tissues was evaluated by calculating the tissue availability (F_t). The studied combination LGM (component mole ratio 1:4:2, respectively) was

prepared at UfIC, RAS [8]. Results were statistically processed using the Statistica program. The obtained data were statistically analyzed by calculating the arithmetic mean (*M*), standard deviation (*SD*), and their confidence intervals (SI L, 95%; SI U, 95%) [9].

RESULTS AND DISCUSSION

HPLC was used to determine LC and its metabolite *N*-DLC (Fig. 1). The retention time of LC was 4.8 min; of *N*-DLC, 8.5 min (Fig. 2). The limit of detection was 0.1 μg/mL; limit of quantitation, 0.2 μg/mL; accuracy, 96.7%. The precision was ≤10% for all concentrations.

The pharmacokinetic profile of the FDF of LGM in blood plasma upon intragastric administration to male rats in various doses showed a phase of increased concentration or absorption during which the maximum blood-plasma concentration was reached. A second part of the pharmacokinetic curve characterized elimination of the drug from blood plasma (a phase of decreased drug concentration) and was finished by the second hour of the study (Fig. 3).

The FDF of LGM was rapidly absorbed from the gastrointestinal tract (GIT). The maximum concentration of the compound was reached by 15 min after administration. The concentration of *N*-DLC increased simultaneously. The pharmacokinetic profile of *N*-DLC also had phases of increased concentration and elimination. The maximum concentration of *N*-DLC was reached 15 min after intragastric administration of the FDF of LGM.

TABLE 1. Pharmacokinetic Parameters of LC and *N*-DLC in Male Rat Blood Plasma upon Intragastric Administration of the FDF of LGM in Various Doses ($M \pm SD$) (SI L, 95%; SI U, 95%)

Parameter	LC	*N*-DLC
	LC in dose 1680 μg/kg (n = 5)	
AUC, μg/mL/h	0.383 ± 0.021 (0.362 – 0.404)	1.059 ± 0.041 (1.016 – 1.102)
K_{el}, h^{-1}	1.183 ± 0.032 (1.149 – 1.216)	0.066 ± 0.005 (0.061 – 0.071)
$T_{1/2}$, h	0.586 ± 0.016 (0.569 – 0.603)	10.625 ± 0.745 (9.843 – 11.407)
MRT, h	0.653 ± 0.033 (0.618 – 0.687)	6.282 ± 0.067 (6.212 – 6.352)
C_{max}, μg/mL	0.931 ± 0.081 (0.845 – 1.016)	0.086 ± 0.005 (0.081 – 0.091)
T_{max}, h	0.250 ± 0.000 (0.000 – 0.000)	0.250 ± 0.000 (0.000 – 0.000)
Cl, L/h/kg	879.241 ± 46.956 (829.963 – 928.519)	317.584 ± 11.978 (305.013 – 330.154)
V_d, L/kg	744.817 ± 59.707 (682.158 – 807.476)	4860.542 ± 226.162 (4623.201 – 5097.885)
	LC in dose 3360 μg/kg (n = 5)	
AUC, μg/mL/h	0.787 ± 0.026 (0.761 – 0.814)	2.102 ± 0.162 (1.932 – 2.272)
K_{el}, h^{-1}	1.228 ± 0.019 (1.207 – 1.248)	0.061 ± 0.004 (0.056 – 0.064)
$T_{1/2}$, h	0.565 ± 0.009 (0.555 – 0.574)	11.542 ± 0.692 (10.815 – 12.268)
MRT, h	0.644 ± 0.025 (0.617 – 0.671)	6.171 ± 0.061 (6.107 – 6.234)
C_{max}, μg/mL	2.323 ± 0.123 (2.196 – 2.453)	0.146 ± 0.011 (0.136 – 0.157)
T_{max}, h	0.250 ± 0.000 (0.000 – 0.000)	0.250 ± 0.000 (0.000 – 0.000)
Cl, L/h/kg	855.498 ± 28.063 (826.048 – 884.948)	321.783 ± 26.103 (294.389 – 349.176)
V_d, L/kg	697.127 ± 31.975 (663.572 – 730.683)	5344.955 ± 322.476 (5006.537 – 5683.373)
	LC in dose 6720 μg/kg (n = 5)	
AUC, μg/mL/h	1.624 ± 0.074 (1.547 – 1.701)	3.039 ± 0.092 (2.943 – 3.137)
K_{el}, h^{-1}	1.255 ± 0.019 (1.23 – 1.276)	0.107 ± 0.003 (0.103 – 0.111)
$T_{1/2}$, h	0.553 ± 0.009 (0.543 – 0.562)	6.488 ± 0.191 (6.288 – 6.687)
MRT, h	0.746 ± 0.029 (0.715 – 0.776)	5.716 ± 0.083 (5.629 – 5.802)
C_{max}, μg/mL	4.882 ± 0.341 (4.524 – 5.241)	0.379 ± 0.028 (0.351 – 0.409)
T_{max}, h	0.250 ± 0.000 (0.000 – 0.000)	0.250 ± 0.000 (0.000 – 0.000)
Cl, L/h/kg	830.094 ± 37.068 (791.193 – 868.995)	443.171 ± 13.545 (428.955 – 457.385)
V_d, mL	661.908 ± 36.962 (623.119 – 700.698)	4150.568 ± 220.203 (3919.479 – 4381.657)

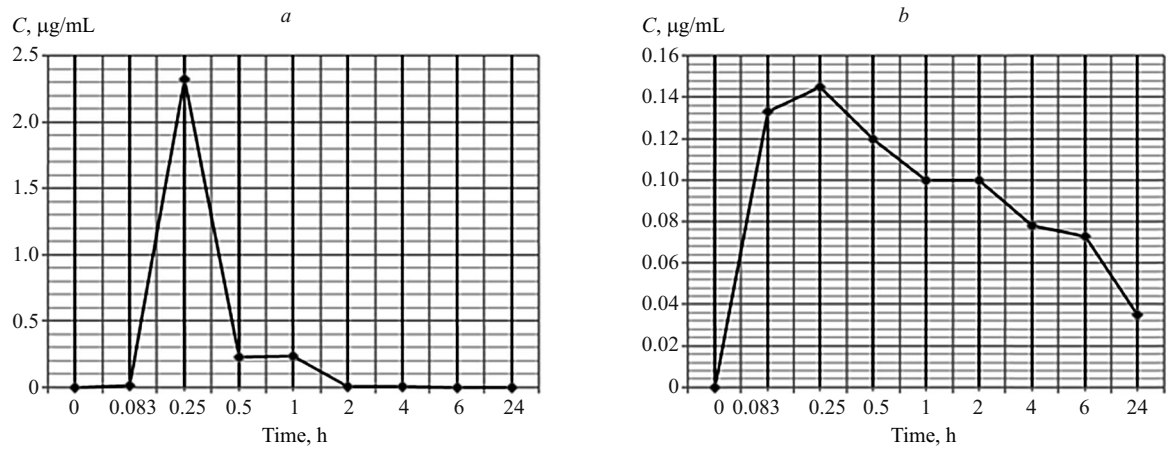

Fig. 3. Pharmacokinetic profiles of LC (a) and *N*-DLC (b) in male rat blood plasma upon intragastric injection of the FDF of LGM (in LC dose 3360 μg/kg).

Table 1 presents the main pharmacokinetic parameters of the FDF of LGM in various doses.

LC was extensively distributed among organs and tissues; exhibited tropism for the kidneys, liver, and myocardium; and was distributed identically in all doses (Table 2).

Studies of the excretion of LC showed that it was detectable in urine for at least 72 h and indicated that a part of it was excreted unaltered by the kidneys.

N-DLC was found in significant amounts in the liver, kidneys, and myocardium (Table 3).

TABLE 2. Pharmacokinetic Parameters of LC Distribution in Studied Doses in Organs and Tissues of Male Rats upon Intragastric Administration of the FCF of LGM ($M \pm SD$) (SI L, 95%; SI U, 95%)

Organ	*AUC*, μg/h/mL	F_t
LC in dose 1680 μg/kg ($n = 5$)		
Liver	1.533 ± 0.049 (1.482 – 1.584)	4.011 ± 0.229 (3.769 – 4.252)
Kidneys	1.139 ± 0.023 (1.115 – 1.162)	2.981 ± 0.199 (2.773 – 3.189)
Myocardium	0.928 ± 0.059 (0.865 – 0.991)	2.429 ± 0.217 (2.201 – 2.656)
LC in dose 3360 μg/kg ($n = 5$)		
Liver	1.951 ± 0.045 (1.903 – 1.997)	2.479 ± 0.101 (2.373 – 2.584)
Kidneys	2.353 ± 0.051 (2.299 – 2.407)	2.991 ± 0.107 (2.879 – 3.103)
Myocardium	1.962 ± 0.056 (1.903 – 2.021)	2.494 ± 0.111 (2.377 – 2.611)
LC in dose 6720 μg/kg ($n = 5$)		
Liver	6.325 ± 0.284 (6.027 – 6.623)	3.906 ± 0.322 (3.568 – 4.243)
Kidneys	7.127 ± 0.455 (6.649 – 7.604)	4.399 ± 0.389 (3.991 – 4.808)
Myocardium	4.119 ± 0.202 (3.907 – 4.331)	2.541 ± 0.171 (2.361 – 2.719)

TABLE 3. Pharmacokinetic Parameters of *N*-DLC Distribution in Organs and Tissues of Male Rats upon Intragastric Administration of the FDF of LGM at Various Doses) ($M \pm SD$) (SI L, 95%; SI U, 95%)

Organ	*AUC*, μg·h/mL	F_t
LC in dose 1680 μg/kg ($n = 5$)		
Liver	0.487 ± 0.019 (0.467 – 0.506)	0.458 ± 0.031 (0.426 – 0.489)
Kidneys	0.322 ± 0.009 (0.312 – 0.332)	0.303 ± 0.018 (0.284 – 0.321)
Myocardium	0.124 ± 0.005 (0.118 – 0.129)	0.117 ± 0.007 (0.109 – 0.124)
LC in dose 3360 μg/kg ($n = 5$)		
Liver	0.993 ± 0.0447 (0.946 – 1.039)	0.476 ± 0.057 (0.416 – 0.535)
Kidneys	0.639 ± 0.021 (0.616 – 0.661)	0.306 ± 0.027 (0.277 – 0.334)
Myocardium	0.263 ± 0.011 (0.251 – 0.275)	0.149 ± 0.059 (0.087 – 0.212)
LC in dose 6720 μg/kg ($n = 5$)		
Liver	2.384 ± 0.096 (2.284 – 2.485)	0.786 ± 0.051 (0.732 – 0.839)
Kidneys	1.352 ± 0.039 (1.309 – 1.393)	0.445 ± 0.017 (0.427 – 0.463)
Myocardium	0.538 ± 0.023 (0.514 – 0.562)	0.177 ± 0.013 (0.164 – 0.191)

Studies of the excretion of *N*-DLC found that it was detectable in urine for 3 d and indicated that most of it was excreted unchanged through the kidneys. The maximum elimination occurred during the first six hours of observation.

Thus, the pharmacokinetic data obtained upon a single intragastric administration in various doses of the FDF of GLM were indicative of rapid absorption and a short elimination half-life and mean retention time *in vivo* of the molecular drug. An analysis of the pharmacokinetic profiles of LC and *N*-DLC in blood plasma, urine, liver, kidneys, and myocardium was consistent with extensive metabolism of LC into the active metabolite that was excreted through the kidneys.

ACKNOWLEDGMENTS

The article was written using results from preclinical studies of the drug based on a combination of LC, glycyrrhizic acid, and methyluracil. The research was conducted in the framework of State Contract for R&D No. 14.N08.11.0068 of Dec. 2, 2015 and was directed by Prof. V. A. Kataev, Chair of the Pharmacy Department, BSMU.

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