A COMPARATIVE STUDY OF THE EXPERIMENTAL PHARMACOKINETICS OF METACIZINE, ETHMOZINE, AND ETHACIZINE IN DOGS

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Ethmozine and ethacizine are the well-known antiarrhythmic drugs belonging to the class of phenothiazine ω-aminoacyl derivatives. Ethmozine provides effective treatment of ventricular arrhythmia and represents a group of relatively safe preparations among the antiarrhythmic drugs, the main disadvantage of which is the comparatively short duration of the effect [1]. Ethacizine is more effective and produces a longer antiarrhythmic action [2, 3], but there were cases of side effects (inhibited cardiac muscle contractility, decrease in arterial pressure in the intact myocardium) of the drug when administered in large doses.

Recently, the new antiarrhythmic drug metacizine, representing a combination of ethmozine and ethacizine, was developed and patented (RF Patent No. 2076711 of 08.07.93), which is available under the trade name Ethmocor (Reg. No. 191637 of 05.02.99). The new drug was designed as a preparation combining the advantages of both active components and minimizing the risk of side manifestations.

The pharmacological investigation [4] showed that the antiarrhythmic effect of the ethmozine – ethacizine combination markedly exceeds that of each individual component in both intensity and duration and in the therapeutic breadth. The optimum ratio of components (ethmozine and ethacizine) from the standpoint of the maximum antiarrhythmic effect was established at 6 : 1. We used this very ratio in the pharmacological investigation of the drug effect in dogs reported below.

The purpose of this study was to determine the experimental pharmacokinetics of metacizine in comparison to those of ethmozine and ethacizine administered separately.

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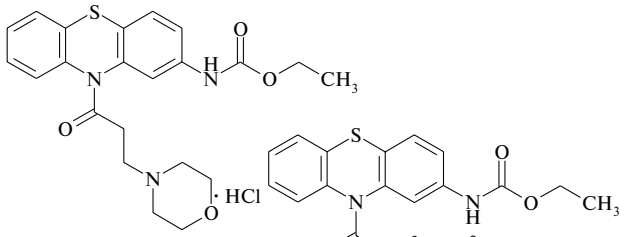
EXPERIMENTAL PART

The experiments were performed with the following substances: ethmozine (etmozin), 2-carboethoxyamino-10-(3-morpholinopropionyl)phenothiazine hydrochloride, 100 mg tablets (Olainfarm, Latvia); ethacizine, 2-carboethoxyamino-10-(3-diethylaminopropionyl)phenothiazine hydrochloride, 50 mg tablets (Olainfarm, Latvia); metacizine tablets containing 150 mg ethmozine and 25 mg ethacizine (Experimental Technology Department, Institute of Pharmacology, Moscow).



Ethmozine (I)

Ethacizine (II)

In addition, we have tested gelatin capsules containing ethmozine and ethacizine, either separately or in the same combination.

The experiments were conducted on mongrel dogs. Each animal received both combined (metacizine) and individual (ethmozine and ethacizine) preparations. Each dog was involved in several series of experiments denoted as follows: A, 1 tablet of metacizine; B, 1.5 tablets of ethmozine; C, 0.5 tablet of ethacizine; D, capsules containing ethmozine and ethacizine taken in the same ratio (150/25 mg) as in metacizine; E, capsules with ethmozine (150 mg); F, capsules with ethacizine (25 mg). In addition, two dogs received a double dose of metacizine (2 tablets) and two other dogs received half of the dose (0.5 tablet). The average values of drug con-

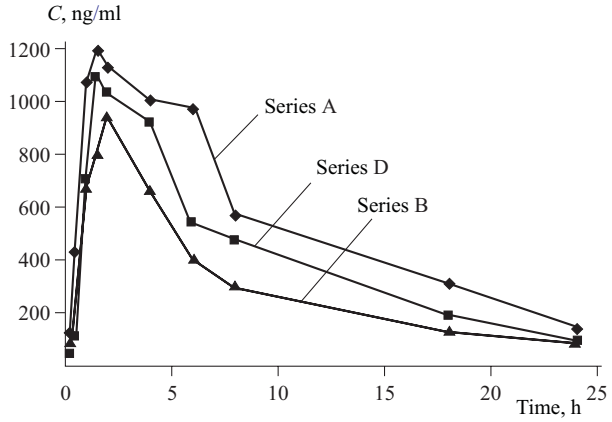


Fig. 1. Average concentration – time profiles of I in the blood of dogs in various experiments.

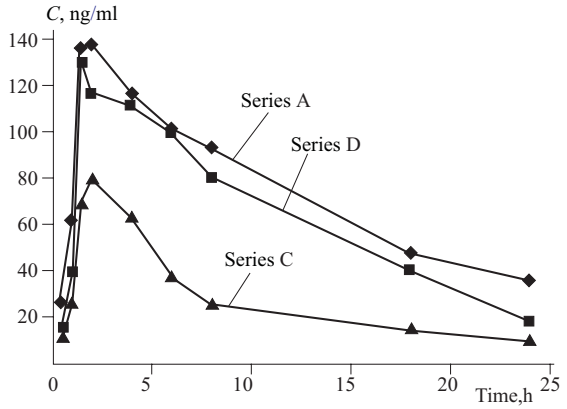


Fig. 2. Average concentration – time profiles of II in the blood of dogs in various experiments.

centrations and the deviations from average were determined in experiments in the following dose ranges (with allowance for the weights of the test animals): I, 7.5 – 12.5 mg/kg; II, 1.2 – 2.1 mg/kg. The time period between different experiments was not less than three days., The time schedule of the tests is presented in Table 1.

The test dogs received drug preparations before morning feed. Control blood samples were taken (20 – 25 min before drug administration) via a catheter implanted into vein. The other samples were subsequently taken 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 18, and 24 h after drug introduction.

The quantitative analyses for ethmozine and ethacizine in the blood serum were performed by HPLC as described in [5, 6]. After the clot formation, the blood samples were centrifuged for 5 min at 3000 rpm. Then the blood serum was separated and stored frozen in a refrigerator (–18°C) until analysis. The samples for HPLC analyses were prepared by the following procedure: to 1 ml of serum were added 20 μl of a chloracyzine solution (internal standard) and 100 μl of 0.05 M NaOH (to adjust pH at 9.0). Then the sample was extracted with 3 ml of a diethyl ether – chloroform (90 : 10 vol.%) and centrifuged at 3000 rpm. The separated extractant layer was mixed with 100 μl of an 0.05 M phosphoric acid solution and the mixture was concentrated by reextraction for 2 min in an Eppendorf mixer. Upon centrifuging, the organic layer was decanted and 10 μl of the aqueous-acid extract was applied onto an HPLC column. The analysis was performed in an SP 8000B chromatograph (Spectra Physics, USA) equipped with an SP 8400 scanning UV detector. The column (Zorbax CN, 250 × 4.6 mm) was eluted with an acetonitrile – phosphate buffer (pH 3.3) – triethylamine mobile phase (35 : 65 : 0.01 vol.%). The drugs were detected at 221 nm; the detection threshold with respect to ethmozine and ethacizine was 5 ng/ml.

TABLE 1. Experimental Schedule and Individual Characteristics of Experimental Animals (Dogs)

Dog	Sex/weight (kg)	Experimental series					
No. 1	M/12	A	0.5A	B	B	D	F
No. 2	M/25	A	2A	B	C	D	E
No. 3	M/14	A	B	C	D	E	F
No. 4	F/10	A	0.5A	B	C	–	–
No. 5	F/11	A	B	C	D	E	F

The pharmacokinetic analysis was conducted using the following program package: M-IND (A. A. Agafonov and V. K. Piotrovskii), ASKID (V. V. Dorokhov and L. E. Kholodov), and COMSTAT. The experimental data were used to determine the following pharmacokinetic parameters: absorption rate constant (k_{01}); area under curve ($AUC_{0-\infty}$); time of attaining maximum concentration (T_{max}); maximum concentration (C_{max}); half-elimination time ($T_{1/2\beta}$); total clearance (Cl_f); mean retention time of drug in the organism (MRT); steady-state distribution volume (V_{ss}).

Subsequent processing of the experimental data yielded AUC/AUC values for both separate drugs and their combination (as a measure of the relative biocompatibility) and C_{max}/AUC (as the absorption rate characteristic).

RESULTS AND DISCUSSION

No side effect related to the administration of metacizine or its components were observed during the experiment. The average concentrations of metacizine components I and II in the blood serum of dogs determined at various time instants after a single drug administration are listed in Table 1. Figures 1 and 2 show the averaged drug concentration profiles in some experimental series. The dynamics of the drug concentration exhibited certain individual variations as well. As can be seen from the data presented in Table 2 and in Fig. 1, the concentration of component I in metacizine (experimental series *A* and *D*) rapidly increases to reach a maximum level within 1.5 – 2 h after administration. Then the concentration of this component gradually decreases (mostly in a two-stage

manner). Administered individually (series *B* and *E*), compound I (ethmozine) reaches a maximum concentration on the average within 2 – 3 h; in the entire time interval studied, the level of this drug in the blood is markedly lower as compared to that upon combined administration (metacizine). A similar pattern is observed (see Table 2 and Fig. 2) for compound II administered with the metacizine composition (series *A* and *D*) and individually (series *C* and *F*). We may also note that some individual pharmacokinetic profiles of compounds I and II exhibited additional peaks (second concentration maximum or plateau) in the 4 – 8 h time interval or even about 18 h after drug administration. These features are also manifested in the averaged profiles. The additional concentration maxima and plateaus may be due to enterohepatic circulation of the drugs. This phenomenon was previously observed in the study of the clinical pharmacokinetics of II [7], but is revealed for the first time with I. In addition to unchanged II, the dog blood serum samples contained a mono-N-deethylated metabolite, frequently in an amount comparable to that of II.

As mentioned above, there were experiments with two pairs of dogs to which metacizine was introduced as in series A but in a double or half dose. The normalized (with an allowance for both doses and animal weights) pharmacokinetic curves of I and II measured in these tests exhibited no significant variations, which indicates that the obtained data are adequately described within the framework of a linear mathematical model.

The combined administration of two pharmacologically active substances may lead to changes in pharmacokinetics

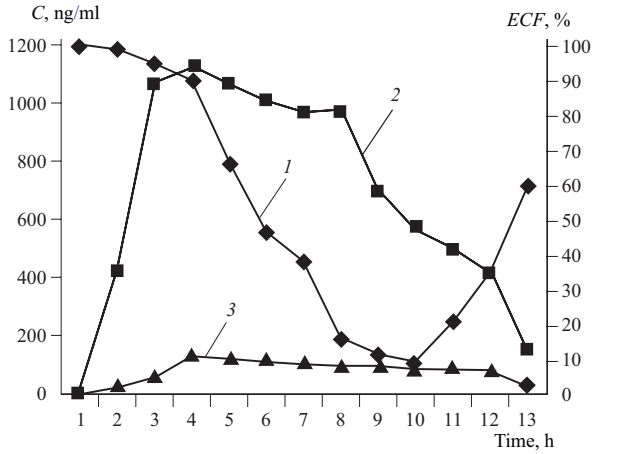


Fig. 3. Relationship between the average concentrations of (*1*) I and (*2*) II in the blood of dogs and the antiarrhythmic effect (*ECF*, %).

in all stages of the drug interaction with the organism, from absorption in the gastrointestinal tract to distribution, biotransformation, and excretion. Interaction between these components may cause changes in the activity of matabolizing enzymes (by stimulating or inhibiting their action). In order to study the interaction of components I and II (and eliminate the possible influence of a difference in the bioaccessibility of metacizine, I, and II), we compared the pharmacokinetic characteristics of these drugs in the form of tablets and gelatin capsules.

TABLE 2. Variation of the Ethmozine (I) and Ethacizine (II) Concentrations in the Blood of Dogs in the Course of Various Experiments

Experiment (p = 95 %)	Drug concentration *, ng/ml									
	Time, h									
	0.25	0.5	1	1.5	2	4	6	8	18	24
I, Series A	126	427	1070	1187	1129	1006	970	571	309	140
n = 6	± 81	± 223	± 523	± 463	± 397	± 382	± 312	± 210	± 97	± 91
I, Series B	92	293	667	792	937	663	401	298	130	77
n = 5	± 67	± 204	± 405	± 328	± 342	± 289	± 213	± 194	± 80	± 39
I, Series D	41	199	795	1089	1035	920	533	490	191	88
n = 5	± 19	± 46	± 273	± 558	± 476	± 325	± 234	± 186	± 69	± 33
I, Series E	29	91	372	546	961	701	511	327	99	47
n = 4	± 23	± 68	± 332	± 431	± 630	± 353	± 219	± 148	± 62	± 33
II, Series A	–	27	61	136	137	116	101	92	47	35
n = 6	–	± 12	± 31	± 89	± 71	± 78	± 67	± 81	± 39	± 32
II, Series B	–	11	27	69	80	63	37	25	14	9
n = 5	–	± 4	± 8	± 26	± 19	± 21	± 9	± 13	± 8	± 7
II, Series D	–	15	39	131	116	111	100	80	41	17
n = 5	–	± 9	± 19	± 62	± 39	± 34	± 28	± 26	± 25	± 11
II, Series F	–	10	32	102	89	84	55	39	23	10
n = 4	–	± 8	± 24	± 52	± 41	± 37	± 32	± 19	± 16	± 8

* Average ± confidence interval.

The averaged pharmacokinetic parameters of metacizine components and the same compounds (I and II) introduced separately are summarized in Table 3. The most difficult task was to interpret data characterizing the stage of drug absorption in the gastrointestinal tract The absorption rate constants k_{01} calculated within the framework of a two-compartment model with absorption were subject to considerable individual variations. The same behavior was typical of the integral parameter C_{max}/AUC representing one of the most reliable characteristics of the drug absorption rate [8]. The large scatter of data is related to uncertainty in calculation of the maximum concentration C_{max} and the time T_{max} required to attain this concentration level. A comparison of the obtained experimental data and the theoretical curves calculated within the framework of the two-compartment model employed showed that this model is insufficiently accurate in reflecting individual concentration – time profiles. The differences between average values of the above pharmacokinetic parameters obtained in various experimental series are statistically unreliable. Nevertheless, considering these values as rough estimates of the drug absorption process, we note that the rate (k_{01}) and extent (C_{max}) of absorption for both components I and II is higher with metacizine composition (series A) than with individual preparations (series B and C, Table 3). An analogous conclusion was made upon a comparative analysis of data for metacizine (series D) and individual compounds I and II (series E and F) administered with gelatin capsules.

The obtained experimental data are more adequately described using a one-compartment model with a nonmodel characteristic of absorption or using a model of statistical moments based on a model-independent approach. For example, the *AUC* values for the pharmacokinetic profiles of I and II administered with metacizine (series A and D, Table 3) are reliably greater than those for the individual drugs taken in both tablets and capsules: $AUC_{I(A)}/AUC_{I(B)} = 1.82$; $AUC_{II(A)}/AUC_{II(C)} = 3.70$. With capsules, the *AUC* values for I and II in metacizine are greater than those for the individual compounds I and II by a factor of 1.44 and 2.01, respectively. This is evidence of a longer exposure of the combined drug components in the organism. The same conclusion is derived from an analysis of the half-elimination time ($T_{1/2\beta}$) and the average time (*MRT*) of drug retention in the organism.

An analysis of the drug concentration dynamics shows that the total clearance Cl_t, which is a measure of the ability to eliminate a given drug from the organism, is lower for I and II in metacizine than for the individual preparations. For compounds I and II in metacizine tablets, the clearance drops on the average by a factor of 1.8 and 3.7, while the Cl_t values in capsules drop 1.45 and 1.65 times, respectively. The steady-state distribution volumes of both I and II also decrease in the case of joint administration (Table 3).

The comparative pharmacokinetic characteristics of tablets and capsules showed that the higher levels of I and II in the blood of dogs treated with combined preparation cannot be explained only by the higher bioaccessibility of metacizine tablets as compared to individual ethmozine and ethacizine tablets. The whole body of data indicate that the components in the combined preparation exhibit a mutual influence in all stages of their circulation in the animal. The interaction most significantly affects the parameters characterizing elimination of the drugs, which may reflect their competition in the interaction with metabolizing enzymes. As expected, this phenomenon is most clearly manifested for ethacizine, the

TABLE 3. Variation of the Pharmacokinetic Parameters of Ethmozine (I) and Ethacizine (II) in the Course of Various Experiments in Dogs

Experiment $p = 95$ %	Pharmacokinetic parameters (average ± confidence interval)								
	k_{01}, h^{-1}	C_{max}, ng/ml	T_{max}, h	*AUC*, ng · h/ml	C_{max}/AUC	$T_{1/2\beta}$, h	V_{ss}, liter	*MRT*, h	Cl_t, liter/h
I, Series A	.09	1207	1.9	14130	0.086	8.16	125	11.6	10.8
$n = 6$	± 1.47	± 419	± 0.7	± 1110	± 0.03	± 1.36	± 15	± 1.2	± 1.1
I, Series B	0.73	780	2.9	7780	0.099	6.24	173	10.1	19.2
$n = 5$	± 0.36	± 187	± 0.6	± 730	± 0.03	± 1.12	± 23	± 1.4	± 1.5
I, Series D	1.68	928	2.4	10110	0.092	6.49	147	10.5	14.8
$n = 5$	± 1.01	± 196	± 0.8	± 950	± 0.03	± 1.43	± 29	± 1.8	± 1.6
I, Series E	1.34	667	3.1	7020	0.094	5.77	179	8.9	21.4
$n = 4$	± 0.63	± 108	± 0.8	± 830	± 0.04	± 1.35	± 31	± 1.5	± 1.9
II, Series A	0.57	127	3.5	2440	0.059	12.6	194	18.9	10.3
$n = 6$	± 0.21	± 82	± 0.9	± 280	± 0.02	± 1.78	± 47	± 2.1	± 1.4
II, Series C	0.60	53	3.1	660	0.081	8.1	396	12.1	37.8
$n = 5$	± 0.27	± 34	± 0.8	± 210	± 0.03	± 1.24	± 52	± 2.3	± 2.8
II, Series D	1.04	103	3.8	1630	0.067	7.25	179	12.4	15.3
$n = 5$	± 0.43	± 49	± 0.9	± 370	± 0.03	± 1.43	± 38	± 1.9	± 2.5
II, Series F	0.91	50	3.9	810	0.061	8.1	314	12.3	25.3
$n = 4$	± 0.61	± 38	± 1.4	± 350	± 0.04	± 1.7	± 39	± 1.8	± 1.6

content of which in metacizine is six times smaller as compared to that in the pure drug.

Examples of such a pharmacokinetic interference of ethmozine with some other drugs were also reported. For example, the combined administration of ethmozine and cimetidine [9, 10] was accompanied by a reliable increase in the concentration of I in the plasma, as was manifested by an increase in the corresponding *AUC* value: $AUC_{\text{I + cimetidine}}/AUC_{\text{I}} = 1.4$. The drug elimination process slowed down: the clearance of I decreased 1.9 times and the half-elimination time increased 1.35 times. However, there were cases where no such interaction took place [10]: the combined administration of I with digoxin only led to a small (15 – 19%) increase in the digoxin concentration, without significantly changing the pharmacokinetic parameters.

The higher level of concentrations of the metacizine components in comparison with the values for their individual preparations can also be partly related to the higher bioaccessibility of metacizine. Taking the bioaccessibility of the reference individual drugs (ethmozine and ethacizine) in tablets as 100%, the relative bioaccessibility f' of ethmozine and ethacizine in metacizine tablets amounts to 182 and 370%, respectively.

The pharmacokinetic behavior of metacizine components is well correlated with the pharmacodynamic data. The ethmozine – ethacizine combination exhibits a higher antiarrhythmic activity and a longer antiarrhythmic effect duration as compared to those of the individual drugs administered separately at the same doses. Figure 3 shows the relationship between concentrations of the metacizine components and the antiarrhythmic effect manifested by a decrease in the ventricular ectopic contraction frequency (*ECF*) in awake dogs 24 h after second-order ligation of the descending branches of the left coronary artery according to Harris. The administration of metacizine decreases *ECF* by 90% or more, while a 50% decrease is observed over a long time period from 3 to 24 h. The individual administration of ethmozine produces a maximum 50% decrease in *ECF* that lasts for a much shorter period from 1 to 4 h.

Thus, the results of our pharmacokinetic investigation showed that combining two pharmacologically active substances, ethmozine and ethacizine, in a 6 : 1 ratio yields a highly effective antiarrhythmic drug metacizine with a safe content of the active agents. The higher efficacy and longer duration of the combined drug action are due to the longer circulation of metacizine components in the organism as compared to that for the individual drugs.

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