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

CLINICAL PHARMACOKINETICS OF ETHMOZINE AND ETHACIZINE IN THE COURSE OF COMBINED ADMINISTRATION

V. L. Beloborodov,[1] E. M. Bugrii,[2] M. A. Zalesskaya,[1] N. A. Tyukavkina,[1] and N. N. Kaverina[3]

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A promising direction in the pharmacotherapy of arrhythmias is based based upon jointly using various drugs so as to combine the advantages of their active components in the treatment of heart rhythm disorders. Within the framework of this approach, a new antiarrhythmic drug metacizine, representing a combination of ethmozine and ethacizine, was developed and patented (RF Patent No. 2076711 of 08.07.93). Metacizine is available under the trade name Ethmocor (Reg. No. 191637 of 05.02.99).

Preliminary pharmacological investigations of metacizine showed that both the intensity and the duration of the antiarrhythmic effect of this ethmozine – ethacizine combination markedly exceed those of each component administered separately [1]. It was established that, from the standpoint of the maximum antiarrhythmic effect and the breadth of antiarrhythmic action, the optimum ratio of ethmozine and ethacizine is 6 : 1. Our previous investigation of the experimental pharmacokinetics of metacizine in dogs [2] confirmed that this ratio of components provides for an increase in the concentrations of both ethmozine and ethacizine in the blood. This was reflected by an increase in the area under the pharmacokinetic curve, the half-elimination time, and the mean retention time, and a decrease in the total clearance. These parameters showed evidence of the competition of two pharmacologically active components of metacizine, which was especially pronounced in the stages of distribution and elimination.

[1] Sechenov Medical Academy, Moscow, Russia;.
[2] Institute of Experimental Cardiology, State Cardiological Research and Production Complex, Ministry of Public Health of the Russian Federation, Moscow, Russia;.
[3] Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia.

The study was aimed at determining changes in the pharmacokinetic behavior of ethmozine and ethacizine jointly introduced into the organism in the ratio corresponding to metacizine in comparison to the known pharmacokinetics of ethmozine and ethacizine administered separately.

EXPERIMENTAL PART

The investigation of ethmozine and ethacizine pharmacokinetics upon their joint administration was performed in a group of eight patients (six males, two females) with the idiopathic form of ventricular premature complex. The patients were aged 18 to 55 (average, 39 ± 4 years) and their body weights ranged within 56 – 91 kg (average, 73 ± 3 kg). All patients were free of organic disorders in the liver and kidney functions and had no signs of cardiac insufficiency, while showing frequent (up to 20551 ± 5260 per day) premature beats of high grade. Three patients in the group previously showed resistance to therapy using various antiarrhythmic drugs administered separately.

Ethmozine and ethacizine in a 6 : 1 ratio were administered in two doses, 300/50 mg and 150/25 mg, which corresponded to 2.1 – 3.9 mg/kg ethmozine and 0.35 – 0.75 mg/kg ethacizine, respectively. In three patients, the pharmacokinetics of ethmozine and ethacizine was also studied upon separate administration. The time interval between the investigation of separate drugs and their combination was not less than three days with 24-h control ECG monitoring. The blood samples for analysis were taken immediately before and 0.25, 0.5, 1, 1.5, 2, 4, 6, 16, and 24 h after drug administration. Prior to the beginning of therapy, all patients

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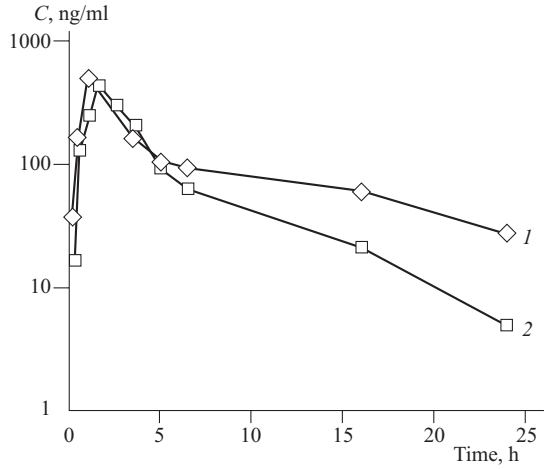

Fig. 1. Average concentration – time profiles of ethmozine in the blood of one patient (B. N.) upon single administration (*1*) in combination with ethacizine and (*2*) separately.

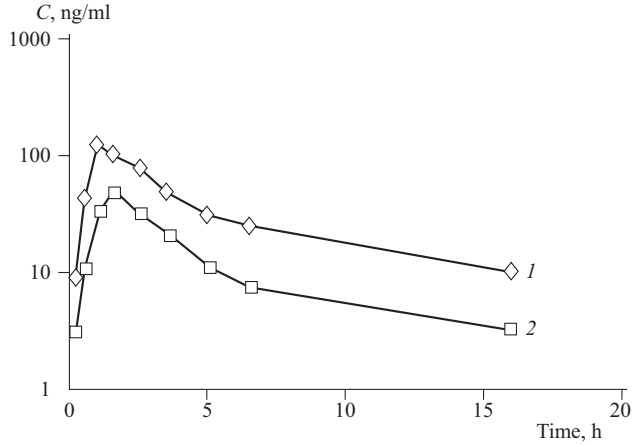

Fig. 2. Average concentration – time profiles of ethacizine in the blood of one patient (B. N.) upon single administration (*1*) in combination with ethmozine and (*2*) separately.

were administered a placebo for 3 – 4 days with subsequent 24-h control ECG monitoring.

The quantitative analyses for ethmozine and ethacizine in the blood serum were performed by HPLC using a specially developed procedure [3]. After the clot formation, the blood samples were centrifuged for 5 min at 2500 rpm. Then the blood serum was separated and stored frozen in a refrigerator (–18°C). 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 solution. 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 8700B 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 233 nm; the detection threshold with respect to ethmozine and ethacizine was 5 ng/ml.

The pharmacokinetic analysis was conducted using the programs M-IND [4] and ASKID [5]. 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_t); mean retention time of drug in the organism (MRT); and steady-state distribution volume (V_{ss}). The drug absorption rate was additionally characterized by the C_{max}/AUC ratio.

The study of pharmacodynamics of the ethmozine – ethacizine combination was supported by clinical observations and by continuous ECG monitoring with a Holter-type system (Del Mar Avionics, USA), followed by retrospective ECG analysis. The criterion of efficacy of the antiarrhythmic therapy was a 70% decrease in the total number of extrasystoles (for pairwise beats, by 90%) and the complete absence of paroxysms of the ventricular tachycardia and ventricular extrasystoles of the R-on-T type.

RESULTS AND DISCUSSION

Figures 1 and 2 show the typical dynamics of ethmozine and ethacizine concentrations in the blood serum of one patient upon combined and separate administration. Both ethmozine and ethacizine are quite rapidly absorbed from the gastrointestinal tract: the concentration of ethmozine in the blood serum reaches maximum 1 – 1.5 h, and that of ethacizine, 2 – 2.5 h after their joint administration. Then, the concentration of each component remains on a high level and gradually decreases for 6 – 16 h after intake; 24 h after administration, ethmozine was detected in the blood serum of all patients and ethacizine – in most of them (for the HPLC sensitivity threshold of 10 ng/ml). The levels of ethmozine and ethacizine in the blood serum upon separate administration was always lower than in the case of joint intake. The blood serum of five patients (of the total of eight) showed the presence of the mono-N-deethylated metabolite of ethacizine in an amount comparable to that of the drug. In two patients this metabolite was not detected, and in one, the maximum concentration was only slightly above the sensitivity threshold. The dynamics of metabolite concentration in the blood serum was subject to considerable individual variation.

The results of processing of our experimental data on the ethmozine and ethacizine pharmacokinetics upon a single

combined peroral administration are presented in Table 1. The pharmacokinetic parameters of ethmozine and ethacizine upon separate administration were determined for three patients and used for comparison with the results of previous investigations. The average values obtained for ethmozine (Table 1) generally coincide with the published data (C_{max} = 360 – 690 ng/ml; T_{max} = 1.16 – 1.78 h; AUC = 1100 – 1890 (ng · h)/ml) [6 – 8], except for a somewhat greater half-elimination time $T_{1/2\beta}$ = 4.9 h (published values fall within 1.3 – 4.3 h) and somewhat lower total clearance Cl_t = 17.3 – 22.4 ml/(min · kg) (published values range from 38.2 to 10.7 ml/(min · kg)). The pharmacokinetic parameters of ethacizine also exhibit no sharp deviations from the values reported for patients with ischemic heart disease [9, 10].

In the case of combined administration, no mutual influence of the two drugs was observed in the stage of absorption (Table 1). The average value of the absorption rate constant k_{01} for ethmozine administered as a metacizine component (0.97 h^{-1}) is very close to the value (1.1 h^{-1}) for ethmozine administered separately (the difference is statistically unreliable). The time to maximum concentration T_{max} and the integral parameter C_{max}/AUC (considered as one of the most reliable characteristics of the drug absorption rate) [11] also reveal no statistically significant differences between ethmozine administered separately and as the metacizine component. On the other hand, the parameters characterizing the duration of drug occurrence in the organism substantially differ. For ethmozine introduced in combination with ethacizine, the area under the pharmacokinetic curve is 1.6 times that for the same drug introduced separately. The half-elimination time and the mean retention time in the former case are also greater by a factor of about 1.5, while the total clearance is accordingly smaller.

Even clearer differences are observed for the other metacizine component, ethacizine. This drug is somewhat more slowly absorbed from the gastrointestinal tract (T_{max} is 1.3 times that for separately administered ethacizine). Similar to the case of ethmozine but much more pronounced (2.9 times) is the growth of the area under the pharmacokinetic curve, at an approximately equivalent drop in the total clearance. Taking into account that the content of ethacizine during the combined administration is six times smaller than that of ethmozine, we may suggest that the latter component is a serious competitor to ethacizine in their interaction with metabolizing enzymes. This competition reduces the rates of metabolism and elimination of ethacizine.

On the whole, we conclude that, in the case of combined introduction of the two antiarrhythmic drugs studied, each component is characterized by a greater time of occurrence in the organism as compared to that of the same drug administered separately. Table 1 also presents data on the pharmacokinetics of an active mono-N-deethylated metabolite of ethacizine, which may also contribute to the antiarrhythmic effect.

The results of our parallel pharmacodynamic and clinical investigations showed that the ethmozine – ethacizine combination produces a stronger and longer antiarrhythmic action as compared to that of the component drugs administered separately in the same doses. Moreover, the therapy was successful in patients previously showing resistance to the therapy using various drugs administered separately. The high clinical effect (90 – 100%) in the suppression of ventricular premature complex was achieved without driving ECG parameters out of the permissible limits.

Our investigation revealed a relationship between drug concentration and therapeutic effect for the jointly administered ethmozine and ethacizine (metacizine). Figures 3 and 4 illustrate this relation by the data for two patients. The effect (percentage decrease in the number of premature beats per hour) develops until complete vanishing of the ventricular premature complex following an increase in the level of ethmozine and ethacizine in the blood serum, reaching a maximum with a small delay after attainment of the maximum drug concentration. Recidivation of the ventricular premature complex appear and grow when the levels of ethmozine and ethacizine decrease below 300 and 50 ng/ml,

TABLE 1. Pharmacokinetic Parameters of Ethmozine and Ethacizine upon Single Combined and Separate Administration

Compound	Pharmacokinetic parameters ($M \pm m$)								
	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
Ethmozine in combination (n = 8)	0.97 ± 0.28	486 ± 150	1.4 ± 0.3	2973 ± 1420	0.18 ± 0.03	7.21 ± 1.54	10.27 ± 4.6	8.9 ± 1.5	18.1 ± 1.3
Ethmozine alone (n = 3)	1.1	427	1.6	1810	0.23	4.9	7.81	6.06	20.2
Ethacizine in combination (n = 8)	0.41 ± 0.14	93 ± 26	2.1 ± 0.7	532 ± 131	0.16 ± 0.03	5.35 ± 0.87	8.24 ± 3.7	7.98 ± 0.9	16.9 ± 4.2
Ethacizine alone (n = 3)	0.49	47	1.7	185	0.25	4.63	16.1	6.59	41.9
Mono-N-deethylated ethacizine (n = 5)	1.31 ± 0.75	101 ± 43	1.2 ± 0.3	353 ± 177	0.27 ± 0.04	2.72 ± 0.74	7.36 ± 4.1	4.56 ± 1.0	26.8 ± 9.3

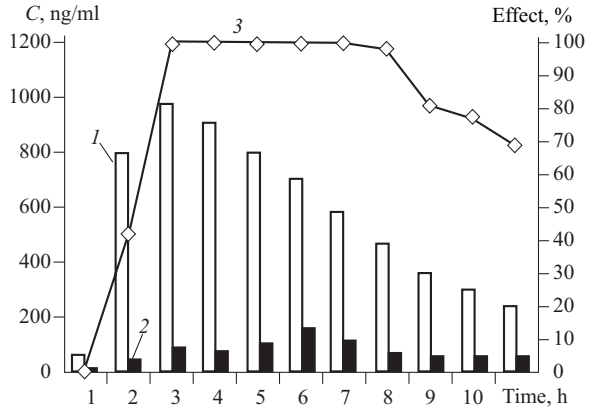

Fig. 3. Relationship between the average drug concentration in the blood of dogs and the antiarrhythmic effect in one patient (R. B.): (*1*) ethmozine level; *n* (*2*) ethacizine level; (*3*) percentage decrease in the number of premature beats per hour.

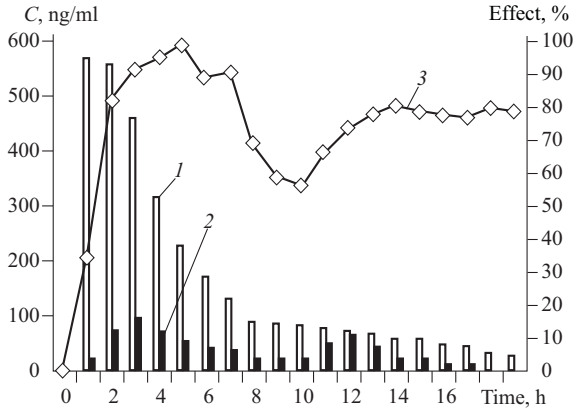

Fig. 4. Relationship between the average drug concentration in the blood of dogs and the antiarrhythmic effect in one patient (S. S.): (*1*) ethmozine level; *n* (*2*) ethacizine level; (*3*) percentage decrease in the number of premature beats per hour.

respectively. A difference in the absorption and elimination rates of and ethacizine favors prolongation of the antiarrhythmic effect. The existence of a relationship between concentrations of the metacizine components and their therapeutic effect is also evidenced by the fact that repeated increase in the ethacizine concentration 10 – 12 h after the first introduction (Fig. 4) led to the expected increase in the antiarrhythmic effect.

Thus, we have demonstrated that combined administration of ethmozine and ethacizine in the optimum ratio 6 : 1 provides for the antiarrhythmic effect with respect to ventricular premature complex of high grade, while not producing any serious side effects upon hemodynamics and cardiac conduction. The levels of ethmozine and ethacizine in the blood serum of patients upon combined administration are always higher than upon separate drug intake in the same doses. The dynamics of drug concentration in the blood serum is adequately reflected by the pharmacokinetic parameters (an increase in $AUC_{0-\infty}$, $T_{1/2\beta}$, *MRT*, and a decrease in Cl_t), showing evidence of prolonged circulation of both ethmozine and ethacizine in the organism upon their joint administration. The results of pharmacokinetic and pharmacodynamic investigation revealed a relationship between the concentrations of ethmozine and ethacizine and the antiarrhythmic effect.

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