Pharmacokinetics and Pharmacodynamics of a New Local Anesthetic Agent

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We compared the effects of local anesthetics procaine, amethocaine (dicaine), bupivacaine, and a new agent RU-1148 on hydrolytic activity of human plasma butyrylcholinesterase. The butyrylcholinesterase-blocking activity of the test substances decreased in the following order: bupivacaine>amethocaine>procaine>RU-1148. The study of the capacity of these agents to form complexes with human plasma proteins and serum albumin showed that RU-1148 in therapeutic concentrations was transported by human serum albumin, β-globulin, and acid glycoproteins. Study of the mechanisms of pharmacodynamic interactions between clonidine and RU-1148 demonstrated good prospects of their combined use.

Key Words: *local anesthetics; butyrylcholinesterase; complex formation; plasma proteins; human platelets*

Many traditional and modern local anesthetics (LA) not always ensure long-lasting analgesic effect and can produce various side effects. A possible approach to creation of new highly active and safe local anesthetics is the search for compounds with unique pharmacokinetic and pharmacodynamic characteristics. It was recently found that a representative of imidaso[1,2-a]benzimidasoles (laboratory code RU-1148) synthesized at Institute of Physical and Organic Chemistry of Rostov State University by Dr. V. A. Anisimova, is characterized, apart from hypotensive and antiarrhythmic effects, by local anesthetic activity [1].

The resistance of LA to hydrolysis catalyzed by butyrylcholinesterase (BCE) and their capacity to form complexes with plasma proteins determine the main pharmacokinetic parameters of these drugs (half-life period, clearance, and distribution volume) [6].

We showed significant potentiation and prolongation of the analgesic effect of LA used in combination with clonidine (α_2-adrenomimetic) which, apart from spinal antinociceptive effect, possesses anxiolytic and sedative activities and produces a positive effect on hemodynamic parameters and perioperative ischemia [7].

Here we compared the effects of known local anesthetics and a new perspective agent RU-1148 on hydrolytic characteristics of human plasma BCE, analyzed interactions of LA with human serum albumin and plasma using the fluorescent probe method, and studied the triggering mechanisms of realization of combined action of LA and clonidine.

MATERIALS AND METHODS

Our study consisted of 3 stages. The aim of stage 1 was to compare the effects of the test agents on hydrolytic activity of human plasma BCE using inhibitory analysis. Neostigmine, a selective cholinesterase inhibitor, served as the reference drug.

Cholinesterase was isolated from the plasma and hydrolytic activity of the resultant enzyme was evaluated as described previously [3]. The number of active centers per ml of isolated BCE was determined by titration with diisopropylfluorophosphate (DFP, a specific BCE inhibitor). The constant of the catalytic

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reaction rate (k_{cat}) was calculated by dividing the maximum rate (V_{max}) by the concentration of active centers. The equilibrium inhibition constant (K_i) was determined by butyrylcholine hydrolysis (15, 30, 60 μM) in the presence of the studied substances. The data were presented in Dixon's coordinates (1/v; inhibitor concentration).

At stage 2 we analyzed the interactions of local anesthetics with plasma proteins using the fluorescent probe method. 1-(Phenylamino)-8-sulfonaphthalene (ANS) probe was used; its fluorescence during the formation of complexes with plasma proteins is sensitive to drugs reacting with them. The study was carried out on human serum albumin (HSA) and whole plasma. Warfarin and quinidine with proven selective tropism to HSA and β-globulin, α_1-acid glycoprotein, respectively, served as the reference drugs [9].

The parameters of ANS fluorescence in HSA solution and human plasma after treatment with the studied agents were measured by the method described by G. E. Dobretsov in 1989 and recorded using an MPF-3 spectrofluorometer (Hitachi) as described previously [4]. The optimal concentrations of protein and ANS were selected in preliminary experiments so that the maximum fluorescence of samples after addition of the studied agents was at least 30% of their initial fluorescence. The probe solution was prepared directly before use from $(ANS)_2Mg2H_2O$ crystal salt. In order to prevent possible hydrolysis of LA under the effects of plasma esterases, 1 mM EDTA and 10 μM DFP (selective paraoxonase and BCE inhibitors) were added to the samples [5].

Stage 3 included evaluation of the mechanisms of LA and clonidine interactions at the level of platelet adrenergic and imidazoline receptors. The substance affinity for "imidazoline type" binding sites was evaluated by their capacity to compete with ^{3}H-idazoxan in the presence of a 500-fold excess of norepinephrine displacing the labeled ligand from the complex with adrenoceptors [8]. The affinity of the compounds to α_{2A}-type binding sites was evaluated by competitive analysis of ^{3}H-idazoxan binding to platelets in the presence of a 500-fold excess of moxonidine (selective ligand of imidazoline receptors).

TABLE 1. Effects of Drugs on Catalytic Activity of Human Plasma BCE (*M*±*m*)

Experiment conditions	K_i, μM	k_{cat}/K_m
Control	—	1375
Amethocaine	15.6±1.3	914
Bupivacaine	14.2±1.5	724
Neostigmine	13.3±0.7	573
Procaine	18.3±1.4	1250
RU-1148	23.4±1.8	1256

Note. Results of 4 independent experiments are presented. Final concentration of substances was 100 μM.

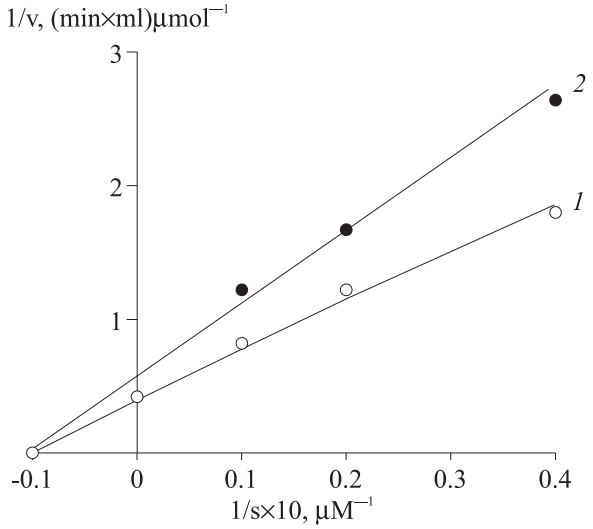

Fig. 1. Relationship between the rate of butyrylcholine enzyme hydrolysis and substrate concentration in the absence (*1*) and presence of RU-1148 (*2*) in Linewiver—Berk coordinates.

In order to differentiate between the LA effects mediated by imidazoline and adrenergic receptors, we used a previously described approach including evaluation of the affinity and intrinsic activity of the compounds towards platelet imidazoline and α_2-adrenoreceptors [2]. Platelet aggregation was recorded using a Biolan device. The effects of the agents on induced modification of light transmission were evaluated by measuring the maximum amplitude (percentage of light transmission increase after addition of the inductor), the time from initiation of aggregation to the maximum light transmission, and the maximum aggregation rate.

The results were processed using Student's *t* test.

RESULTS

BCE activity estimated by hydrolysis of 1 mM butyrylcholine was 21 U/ml (μmoles of substrate hydrolyzed over 1 min at 37°C was taken for a unit of enzyme activity). The resultant volume of homogenous BCE was sufficient for characterization of molecular kinetic of the enzyme. Addition of EDTA (paraoxonase inhibitor) to samples did not modify hydrolysis of butyrylcholine and test substances in the plasma. On the other hand, activity of the isolated enzyme decreased by 74% in the presence of 0.1 M NaF, which is in line with published data (inhibition of BCE in the presence of fluorine ions is typical of this enzyme) [10].

The parameters of catalytic activity of BCE isolated in our study were K_m=10.4±1.1 μM (Michaelis

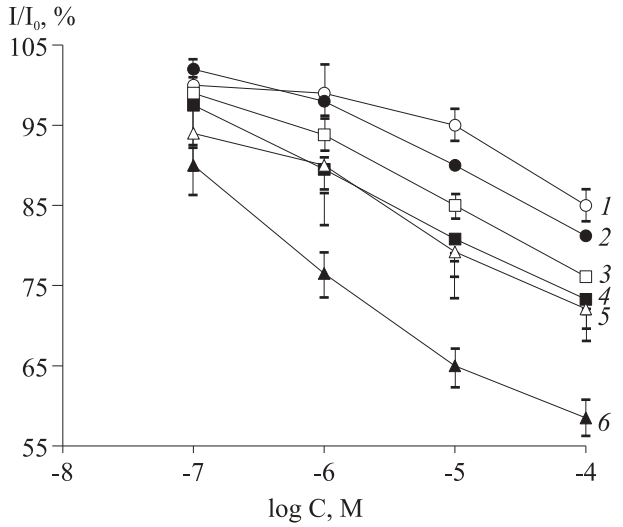

Fig. 2. Effects of procaine (*1*, *4*), quinidine (*2*, *5*), and lidocaine (*3*, *6*) on parameters of ANS fluorescence in human serum albumin solution (*1-3*) and human plasma (*4-6*). Here and in Fig. 3: abscissa: logarithm of final concentrations of test agents in the incubation medium; ordinate: relative changes in ANS fluorescence (I/I_0) in percent of control (no drugs).

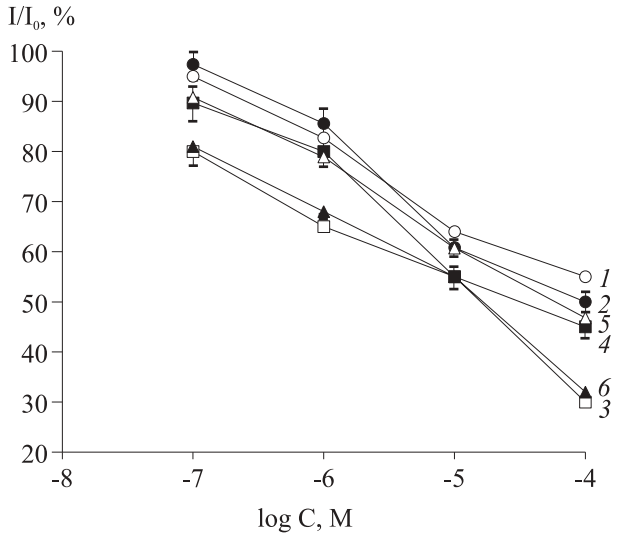

Fig. 3. Effects of amethocaine (*1*, *4*), RU-1148 (*2*, *5*), and warfarin (*3*, *6*) on parameters of ANS fluorescence in human serum albumin solution (*1-3*) and human plasma (*4-6*).

constant of enzyme reaction), V_{max}=2.4±0.3 mmol/min, k_{cat}=14,300 min^{-1}, and k_{cat}/K_m=1375 (catalytic efficiency).

The inhibition constant (K_i) was calculated by the method of Dixon, the values of catalytic efficiency of BCE were summed up and estimated in the presence of the agents in a concentration of 100 μM and in the absence of the test substances (Table 1).

For evaluation of the type of BCE inhibition in the presence of RU-1148 the data of inhibitory analysis were presented in inverse Lineweaver—Burk coordinates (Fig. 1). By the mechanism of inhibition RU-1148 is a noncompetitive inhibitor of the enzyme, that is, it does not modify enzyme affinity for the substrate (K_m), but decreases the number of "active" functioning catalytic centers of BCE. It can be hypothesized that RU-1148 is not hydrolyzed by BCE, because it is not complementary to the enzyme active center.

These data indicate that LA capacity to modulate hydrolytic activity of BCE decreases in the following order: bupivacaine>amethocaine>procaine>RU-1148. Hence, by the mechanism of inhibition procaine is a competitive inhibitor of the enzyme, bupivacaine and amethocaine are characterized by mixed type of inhibition, and RU-1148 is a noncompetitive inhibitor of the enzyme.

The drugs were divided into two groups by the results of measurements of ANS fluorescence in HSA solution and whole plasma (Figs. 2, 3). Group 1 included quinidine, procaine, and lidocaine in concentrations of 0.1-1 μM, which virtually did not change the intensity of ANS fluorescence in HSA solution and decreased it in the whole plasma. In concentrations of 10-100 μM these drugs interacted with HSA.

Group 2 included RU-1148, amethocaine (dicaine, tricaine), bupivacaine, and warfarin decreasing fluorescence intensity in both HSA solution and whole plasma. Similar changes in fluorescence in the objects for warfarin indicates that these agents are bound predominantly by the nonspecific transporting system of the blood (HSA). Amethocaine, bupivacaine, and RU-1148 more intensely decreased the intensity of ANS fluorescence in whole plasma than in the ANS+HSA complex. The results indicate that these substances are transported both by the nonspecific transporting system and by β-globulin and acid glycoproteins.

The data on the relative affinity of the studied agents for platelet imidazoline and α_2-adrenoreceptors are summed up in Table 2. In contrast to clonidine, amethocaine in concentrations of 0.1-100 μM (including fluctuations of its therapeutic concentrations in

TABLE 2. Competitive Analysis of Binding of the Studied Compounds to Platelets (IG_{50})

Conditions	Clonidine	RU-1148	Amethocaine
^{3}H-idazoxan+norepinephrine	175 nM	394 nM	>10^{-4} M
^{3}H-idazoxan+moxonidine	68 nM	4.6 μM	>10^{-4} M

Note. The inhibitor concentrations IC_{50} are presented. Final concentration of labeled idazoxan was 40 nM, duration of incubation 2 h at 4°C.

TABLE 3. Effects of Studied Compounds on Parameters of ADP-Induced (0.25 μM) Platelet Aggregation (*M*±*m*)

Experimental conditions		Maximum amplitude of aggregation, %	Maximum rate, arb. units	Time of aggregation, min
Control (0.25 μM ADP)		23.5±2.0	144±15	3.8±0.2
Amethocaine	2 μM	22.4±2.3	141±15	3.7±0.1
	5 μM	25.3±2.0	145±12	3.9±0.2
Clonidine	2 μM	31.1±2.7*	193±14*	3.3±0.1
	5 μM	33.7±2.6*	209±14*	3.1±0.1
RU-1148	2 μM	21.8±1.3	137±11	3.5±0.2
	5 μM	19.5±1.2*	108±16*	3.7±0.1

Note. Data of 3-5 independent experiments are presented. *$p<0.05$ compared to the control.

the plasma) did not bind to receptors. On the other hand, RU-1148 effectively competed for imidazoline binding site and demonstrated far lower affinity for α_{2A}-receptors.

For evaluation of pharmacodynamic properties of RU-1148 we studied its effect on ADP-induced platelet aggregation. This test system allows discrimination of chemicals by their relative capacity to activate or inhibit imidazoline and α_2-adrenoreceptors (Table 3). RU-1148 in a concentration of 100 μM significantly reduced parameters of platelet aggregation, which can be due to activation of imidazoline receptors under the effect of this agent, presumably predominating over stimulation of platelet adrenoceptors. Preferential binding of clonidine to α_{2A}-receptors (the ratio of binding activity to adrenergic/imidazoline receptors is 16) explains its potentiating effect on ADP-induced aggregation. The results indicate that new anesthetic RU-1148 binds to and activates imidazoline receptors, while the possibility of potentiation of the effects mediated by α_{2A}-adrenoreceptors by this substance in therapeutic concentrations is extremely low.

Hence, the pharmacodynamic interaction in the RU-1148+clonidine combination can be explained by the fact that both agents activate imidazoline receptors.

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