Conformation and Kinetic Characteristics of Interactions between Local Anesthetics and Aqueous Solutions of Hydroxypropylmethylcellulose

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Conformation and kinetic characteristics of the interactions of local anesthetics lidocaine (xycaine), tetracaine (dicaine), bupivacaine, and new RU-1117 compound with proven anesthetic activity with Visiton (1% hydroxypropylmethylcellulose in phosphate buffer) were studied. It was found that complex formation between the local anesthetics and hydroxypropylmethylcellulose is a time-dependent reversible process. The equilibrium is attained within 2.5-8.0 h and depends on the chemical nature of local anesthetic.

Key Words: *hydroxypropylmethylcellulose; local anesthetics; interaction kinetics*

Hydroxyalkyl (hydroxypropylmethyl-, hydroxyethyl-) cellulose derivatives (HC) similar by their chemical properties are widely used in various aqueous systems as stiffeners and stabilizers. The use of HC in pharmaceutical industry as a filler for solid dosage forms is based on the capacity of HC to regulate drug release. The molecules of HC polymer form complexes with drugs, similar to the ligand-receptor complexes [6]. The formation of reversible HC—drug complex provides the basis for creation of slow release dosage forms.

The HC—drug interaction can lead to cation-induced changes in the adsorption and conformation properties of HC. It was established that addition of drugs and electrolytes (sodium or calcium salts as obligatory components of the solvents) to aqueous solutions modifies the adsorption and rheological characteristics of the polymer [4]. These changes are explained by conformation restructuring of polymer moieties, primarily by their mobility. The interaction of cations with the polymer leads to aggregation of polymer molecules as a result of the increase in the number of hydrogen bonds and hydrophobic interactions. In low concentrations cations form microspheres in water solutions; this increases polymer solubility and polarity [9]. In turn, this induces intermolecular aggregation of the polymer and increases hydrophobic properties of HC [13]. High concentrations of electrolytes impair the stabilization characteristics of hydrophilic polymer solutions, cause relaxation of HC molecules, and modulate their tertiary structure. These conclusions were made from studies of "dynamic surface tension" of HC solutions [6]. For example, electrolytes increase the dynamic surface tension associated with achievement of the equilibrium. The initial increase in dynamic surface tension is due to the effect of the electrolyte on solution polarity, while its decrease is due to the formation of hydrophobic clusters in HC. This leads to reduction of solvent diffusion, manifestation of the resistance to polymer mobility and increase of its viscosity [11]. The capacity of the polymer to bind drug molecules is more important for the regulation of drug release than cation-induced mo-

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dification of solution viscosity. Critical concentration of the drug (usually within 0.05-5%) serves as a quantitative characteristic of polymer adsorption; this concentration reflects balanced dynamic status of the system, when the probabilities of the formation of polymer—polymer and polymer—drug complexes are equal [10]. The more cations added into the system, the higher is polymer liability to condensation and reduction of solution viscosity. The decrease in fluidity is associated with loss of adsorption properties of the polymer, displacement of the drug into solution, and increased drug release [9].

The aim of our studies was to clear out the mechanisms of interactions of local anesthetics (LA) with the blood transport systems, *e.g.* human serum albumin (HSA); the results can be used for prediction of the pharmacokinetic and pharmacodynamic characteristics of LA: the degree and duration of the anesthetic effect, rate of metabolism and/or utilization in peripheral tissues [1,2].

Experimental and clinical data on the use of LA based on Visiton (1% HC solution in phosphate buffer) indicate the necessity of choosing the effective LA concentration with consideration for the "incubation period": the efficiency of anesthesia depends on the duration of preliminary co-incubation of LA and HC before their direct application *in vivo*. However, kinetic parameters of the HC—LA system were not estimated; only preliminary qualitative studies were carried out.

We studied the type and parameters of interactions between known LA and RU-1117, a prospective new compound with proven anesthetic activity, and Visiton preparation *in vitro*.

MATERIALS AND METHODS

Visiton, a preparation manufactured by Eye Microsurgery Center, has the following composition: 1% HC, 0.85% sodium chloride, 0.144% sodium hydrophosphate, and 0.03% sodium dihydrophosphate, pH 7.2.

For evaluation of the effects of LA on conformation characteristics of 1% HC (Visiton), optical density of solutions at λ=320 nm (OD_{320}) was measured on a Hitachi P400 spectrofluorometer. In preliminary experiments, the time required for attaining equilibrium in the HC—LA system was found: 2-8 h depending on the type of LA.

Tritiated local anesthetics were prepared by the universal method developed for introduction of radioisotope label in tertiary amines [5]. Specific activity of the resultant preparations was 54±10 Ci/mol.

The kinetic parameters of interactions between HC and ^{3}H-LA (direct effect) and LA-binding activity of HC in the presence of an excess of unlabeled LA (reversibility) were studied at pH 7.2 and 22±1°C by the method based on the use of dextran-coated charcoal with consideration for background binding (without HC in the analytical system) [3]. The concentrations of HC and LA were chosen to provide specific binding of 40-50% labeled anesthetic introduced into the incubation medium.

The results were processed using Pharmacological Basic Statistics software. Confidence intervals for experimental values and the significance of differences were evaluated by Student's test at a significance level 0.05.

RESULTS

Addition of ascending concentrations of LA to Visiton was accompanied by two closely linked processes. Ionic strength of the solution increased due to electrolyte characteristics of LA (the greater part of LA molecules are in the cationic form at pH 7.2), the polymer conformation changed, the molecules were unfolded and drug-binding hydrophobic clusters were lost, the solution viscosity decreased, and film structures formed. These changes led to an increase in the optical density of the solution (including that at the expense of light scatter). Increasing LA concentration increases the probability of HC—LA complex formation. Hence, the critical concentration of LA (when the content of polymer—LA and polymer—polymer complexes is equal) is determined by individual physicochemical (electrolytic) and stereochemical characteristics of LA.

For evaluation of the effect of LA on conformation characteristics of HC and selection the "critical concentration" of LA (at which optical density of HC solution increased by 50%), we studied changes in optical density of Visiton after addition of ascending concentrations of LA (0.01-5%). Sharp increase in light scatter indicated saturation of HC binding sites and conformation changes in the polymer induced by addition of high concentrations of LA (Fig. 1). According to the means of three independent experiments, the "critical concentration" in the LA—HC system was 1.7±0.2% for lidocaine, 0.08±0.02% for RU-1117, 0.40±0.05% for tetracaine, and 0.9±0.1% for bupivacaine. The observed appreciable differences in the critical concentrations of LA can be caused by individual chemical structure of LA, for example, pKa value. pKa value (pH value at which the concentrations of ionized and nonionized forms of local anesthetic are equal) is a basic factor determining pharmacokinetic and pharmacodynamic characteristics of LA.

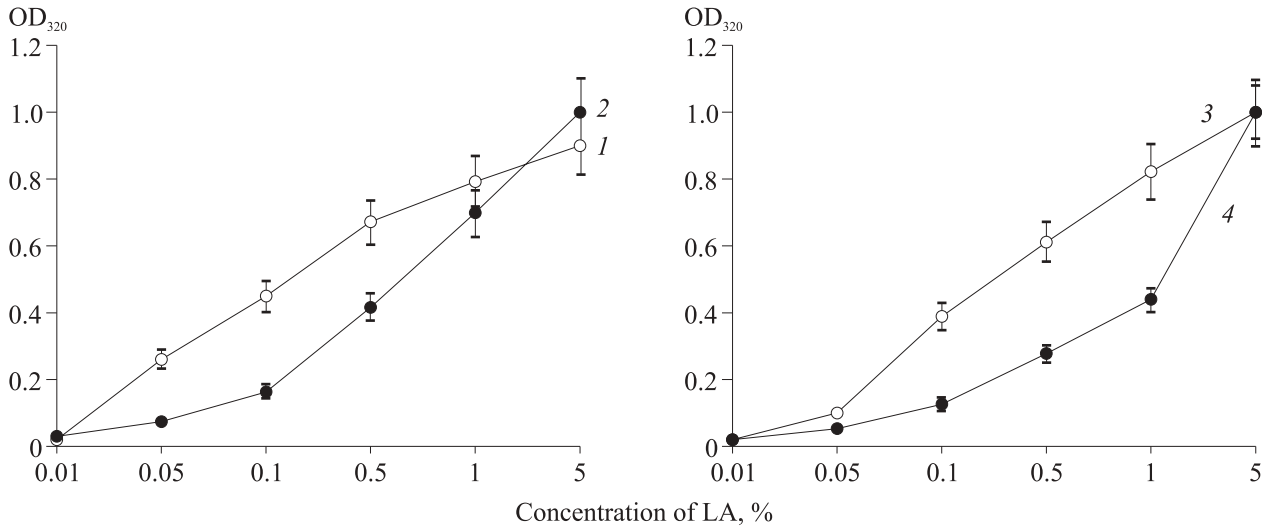

Fig. 1. Effect of ascending LA concentrations on optical density of 1% HC solution. *1*) RU-1117; *2*) bupivacaine; *3*) dicaine; *4*) lidocaine.

The ratio of these two forms depends on their pKa and pH of body fluids in accordance with the Henderson—Hasselbach equation:

$$\log \frac{\text{Cationic form}}{\text{Nonionized form}} = \text{pKa-pH}.$$

Since pKa of the majority of LA is 8.0-9.0, the greater part of these drugs are present in the ionized cationic state in liquid body media. When pH decreases (*e.g.* pH 7.2 during inflammatory processes in tissue), the proportion shifts towards cations and formation of positively ionized compounds from neutral molecules. When pH increases, an opposite process takes place [5].

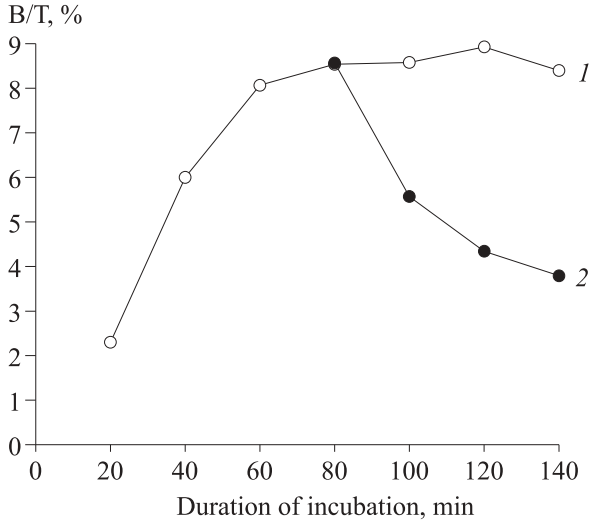

Fig. 2. Kinetics of ^{3}H-dicaine—HC complex formation and dissociation of the complex at 22°C. *1*) relationship between the percent of bound anesthetic (*B/T*) and duration of incubation; *2*) displacement of ^{3}H-dicaine from the complex with HC by unlabeled dicaine excess.

In our case, pH of HC solution is 7.2, which seems to essentially modify the conformation and adsorption characteristics of the HC—LA complex. The fact that the percentage of nonionized LA injected into tissues with pH 7.4 decreases in proportion to pKa for this LA supports this hypothesis. For example, 65% ionized and 35% nonionized molecules of lidocaine (pKa 7.78) are detected in tissue after its injection with pH value of 7.4 [7]. For tetracaine (pKa 8.4) this ratio is 95 and 5%, respectively.

Local anesthetics are weak bases. In clinical practice they are used in the form of salts improving solubility and stability of solutions. Hence, when choosing the effective concentration of LA on the base of Visiton, we should take into consideration high electrolytic characteristics of LA solutions.

We previously studied the equilibrium characteristics of HSA—LA interactions using a fluorescent probe (1-anilinonaphthalene-8-sulfonate (ANS) [2]. The LA—HSA complex formation decreases in the following order: bupivacaine>Ru-1117>tetracaine>>lidocaine>dicaine. Practical use of this method is based on the dependence of ANS fluorescence on the charge, rigidity, and conformation at the site of its binding.

On the other hand, the use of fluorescent probes for evaluation of kinetic parameters of LA and HC interaction is impossible. The radioligand methods quantitatively evaluating the binding of ^{3}H, ^{14}C, or ^{125}I labeled compounds are more often used for this purpose. Introduction of carbon isotope into LA molecule is possible only at the stage of its chemical synthesis, and therefore the use of ^{14}C li-

gands ensures maximum reproducibility of results; on the other hand, this method is most expensive. Introduction of iodine isotope improves the sensitivity of the method, but can modulate the complex-forming characteristics of the ligand [8]. We selected LA modification by introducing a radioactive hydrogen isotope (^{3}H).

The time course and reversibility of complex formation between HC and ^{3}H-LA were studied for all LA. Typical kinetic curves are shown as exemplified by ^{3}H-dicaine binding to HC (Fig. 2, curve *1*). The abscissas of experimental points correspond to total duration of incubation at 22°C and on the cold. Only the duration of "warm" incubation varied in the experiment; subsequent cooling of the system for stabilization of the complex before treatment with charcoal always took 10 min. Curve *2* in Fig. 2 characterizes dissociation of ^{3}H-dicaine—HC complex, caused by introduction of unlabeled LA excess into the system after equilibrium was attained. The kinetic characteristics of HC complex formation with different LA are presented (Table 1).

The time needed for attaining the equilibrium varied significantly for LA of different chemical nature. The minimum values were observed for lidocaine, the maximum for tetracaine and bupivacaine. The new compound RU-1117 is characterized by intermediate values (Table 1). The complex half-dissociation values, determined in our studies, were sufficiently close for all studied LA. It seems that this fact reflects relatively low affinity of HC for LA: according to published data, the equilibrium dissociation constant for LA—HSA complex varies from 3 μM (bupivacaine) to 25 μM (lidocaine) [14].

The data indicate significant changes in the kinetics of HC—LA complex formation and can be used for experimental validation of the procedure for making preparations based on the mixture of HC and LA solutions.

TABLE 1. Kinetic parameters of LA Complex Formation with 1% HC Solution ($M \pm m$)

Drug	Kinetic characteristics	
	time for attaining equilibrium, h	half-dissociation time, min
Lidocaine	2.5±0.1	22±3
RU-1117	4.2±0.3	30±5
Tetracaine	6.5±0.5	27±4
Bupivacaine	8.0±0.4	42±5

Note. Mean values from three independent experiments are presented.

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