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SOME ASPECTS OF THE USE OF LIPOSOMES TO STORE NEUROTROPIC DRUGS

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Administration of biologically active substances to animals in liposomal form has a marked effect on many pharmacologic parameters, such as the dose—effect relationship, toxic and side effects, pharmacokinetics and pharmacodynamics, and so on [2-4].

The degree and site for binding of molecules of neurotropic drugs of the liposomal membrane depend on the electrostatic and hydrophobic interactions. The strength of binding of these molecules with liposomes can be estimated by means of the nuclear magnetic resonance (NMR) method.

The line width of ^{1}H-NMR of amphiphilic substances in the composition of the phospholipid membrane, because of the reduced mobility of their molecules, is much greater than the line width of molecules in the aqueous phase. In the case of rapid exchange between the membrane-bound and free states the molecules of these substances will have a certain intermediate line width, proportional to the coefficient of distribution between membrane and water [1].

The use of this method to determine the strength of binding of molecules of neurotropic drugs with the lysosomal membrane has been made much easier by the fact that the aromatic region of the ^{1}H-NMR spectra of natural phospholipids is transparent, and molecules of most preparations used contain aromatic groups [1]. It is an interesting fact that the molecules of most compounds contain tertiary amino groups, which carry a positive charge at physiological pH values. Addition of negatively charged phospholipids to neutral phospholipids significantly increases the membrane — water partition coefficient for these compounds [6, 7]. Besides the phospholipid composition of the liposomes, their structural organization also affects the rate of release of the preparations. For instance, small single-layered sonicated liposomes ought to give up the preparations faster than large, stratified liposomes of the same composition. By modifying the above parameters, it is possible to vary at will the degree and character of interaction of preparations with liposomes, and thus to act together on their pharmacokinetics.

The aim of this investigation was to study the ability of liposomes to prolong the action of neurotropic drugs when administered locally.

EXPERIMENTAL METHOD

Liposomes were prepared from egg phosphatidylcholine obtained from Khar'kov Bacterial Preparations Factory and azolectin, obtained from "Sigma" (USA). The therapeutic preparations were used in the form of pharmacopoeial

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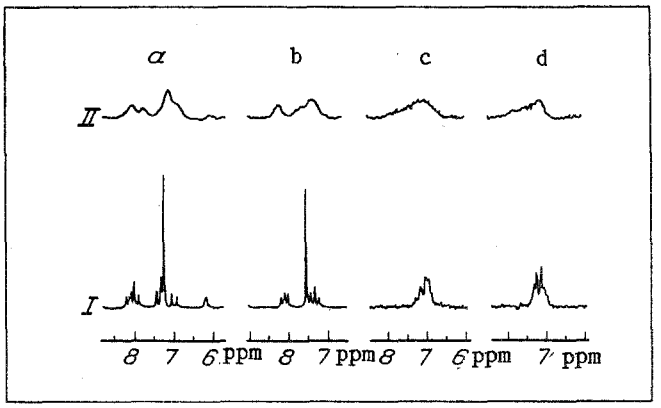

Fig. 1. Aromatic regions of ^{1}H-NMR spectra of droperidol (a), haloperidol (b), chlorpromazine (c), and pipolfen (d) in buffer (^2H_2O, 0.01 M Tris-^{2}HCl, p^{2}H 6.5; 0.1 M KCl) in the absence (I) and presence (II) of sonicated liposomes from egg phosphatidylcholine. Ratio by mass of preparations to phospholipid 1:10. ppm) Parts per million.

solutions (chlorpromazine, amethocaine, procaine, and trimecaine were of Soviet origin; haloperidol, droperidol, pipolfen, and lidocaine were of Hungarian origin; marcain was obtained from the firm of "Bofors, Nobel-Pharma," Sweden).

Liposomes for the NMR experiments were prepared as follows. To the dried phospholipids (egg phosphatidylcholine was dried on a rotary evaporator) was added a buffer of the following composition: (^2H_2O; 0.01 M Tris-^{2}HCl, p^{2}H 6.5; 0.1 M KCl), and the mixture was shaken to obtain a homogeneous emulsion. The emulsion was then aerated for 15 min with argon and treated on a UZDN-1 apparatus with ultrasound at a frequency of 22 kHz for 15 min. Before addition to the liposomes, the preparations were freeze-dried.

To prepare liposomal forms of the preparations for tests on animals, pharmacopoeial solutions of these preparations were added directly to the dry phospholipids. Single-layered (sonicated) liposomes were prepared in the same way as the liposomes for the NMR experiments. Stratified (not sonicated) liposomes were not treated with ultrasound. The ^{1}H-NMR spectra were photographed under Fourier-transform conditions on an FT-80 spectrometer ("Varian," USA) at a frequency of 80 MHz.

Tests of the liposomal forms of the preparations were conducted on albino mice weighing 25-30 g and rabbits weighing 2.5-3.5 kg. Investigations of the duration of catalepsy were conducted on four groups of mice, with 16 animals in each group, except that for determining mortality from the liposomal forms of amethocaine, 20 mice were used in each group. Experiments to determine the duration of peridural anesthesia were conducted on four groups of rabbits, with eight rabbits in each group.

Access to the peridural space in the rabbit was obtained, not in the traditional way through the ligaments of the spine, but by drilling a burrhole in the vertebral arch. A catheter with a guard to prevent trauma to the spinal cord was securely fixed in the bone. The efficacy of the anesthesia produced by the peridural method was assessed from changes in the thresholds of pain sensation during electrodermal stimulation (EDS) of the rabbit's hind limb, on the basis of respiratory, motor, and vocal reactions. EDS was applied through needle electrodes from an MSE-3R electrostimulator ("Nihon Khoden," Japan) at a frequency of 100 Hz, pulse duration 1 msec, within the range 1-100 V. The duration of stimulation was 1-2 sec. The time course of respiration was recorded on an eight-channel polygraph ("Polyrecorder Model R35 gt," from "Galileo," Italy).

To determine the significance of differences in the experiments on animals, Rosenbaum's nonparametric test was used.

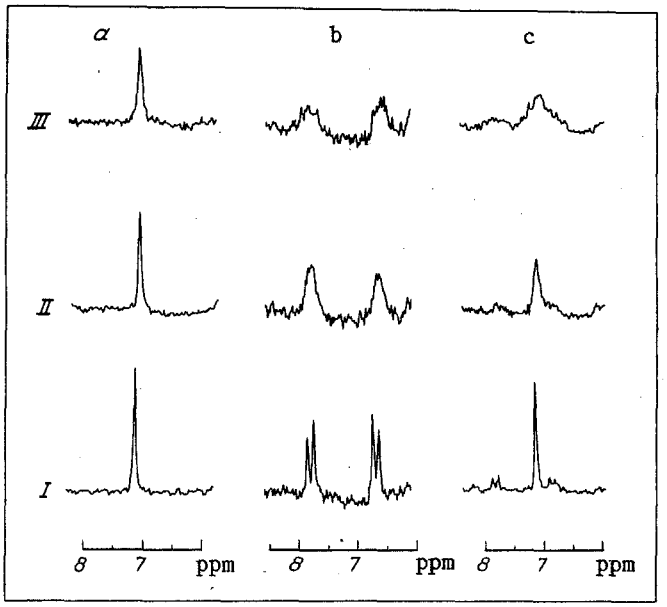

Fig. 2. Aromatic regions of ^{1}H-NMR spectra of lidocaine (a), amethocaine (b), and marcain (c) in buffer (^2H_2O; 0.01 M Tris-^{2}HCl, p^{2}H 6.5; 0.1 M KCl) in absence (I) and presence of sonicated liposomes prepared from egg phosphatidylcholine (II) and azolectin (III). Ratio of preparations to phospholipids by weight 1:10.

EXPERIMENTAL RESULTS

Lines of protons of the aromatic rings of chlorpromazine, pipolfen, haloperidol, and droperidol, in the presence of liposomes from egg phosphatidylcholine, were much wider than lines for preparations dissolved in water, evidence of the firm binding of their molecules with the liposomal membrane (Fig. 1).

A significant influence on the strength of binding of molecules of the drugs with the membrane is exerted by its phospholipid composition. For instance, molecules of amethocaine and marcair bind with azolectin liposomes, with about 20% of negatively charged phospholipids in their composition, better than with liposomes from egg phosphatidylcholine, a neutral phospholipid (Fig. 2). Lidocaine, because of the insufficient hydrophobicity of its molecule, binds weakly with both membranes.

On the whole the results of the NMR tests showed that many neurotropic drugs have high affinity for the liposomal membrane, and for that reason, highly effective liposomal forms of them can be prepared. The most promising form of their use is by local application. In this case liposomes, which remain for a long time at the site of administration, do not break down but form a "depot" of the preparation injected together with it. Besides a marked increase in the duration of action of the injected preparation, liposomes may also significantly reduce its toxicity, for they are bound to lower the excessive concentration of the drug (Fig. 3).

To assess the prolonging action of liposomes we studied the duration of catalepsy in four groups of albino mice. The first two groups received an intraperitoneal injection of haloperidol and droperidol in a dose of 25 mg/kg. The duration of catalepsy in this case was 87 ± 40 min for haloperidol and 59 ± 33 min for droperidol. The animals of groups 3 and 4 received the same drugs intraperitoneally in the same doses, but in single-layer liposomes prepared from egg phosphatidylcholine (ratio of phospholipid to drugs by weight 10:1). In this case the duration of catalepsy was 288 ± 110 min for haloperidol and 133 ± 60 min for droperidol. Thus the liposomes prolonged the action of haloperidol by 3.3 times ($p < 0.01$) and of droperidol by 2.3 times ($p < 0.01$). After injection of haloperidol in the pure form, all 16 mice developed catalepsy after a short but distinct seizure, whereas after injection of the liposomal form, no seizures were observed. This result is evidently due to the steadier rise of the blood level of the preparation when injected in the liposomal form.

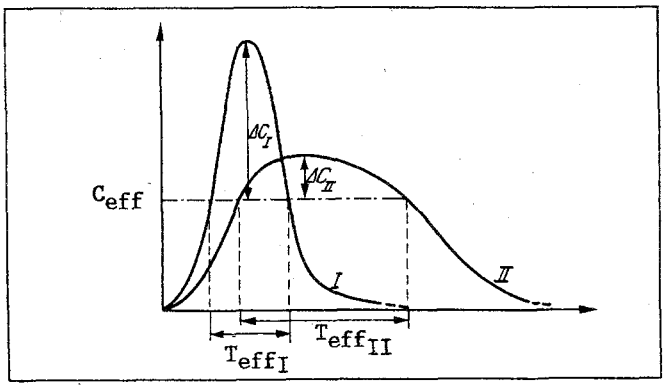

Fig. 3. Hypothetical changes in concentrations of drugs at the site of their action when injected in the pure form (I) and in the liposomal form (II). Abscissa, time; ordinate, concentration of drugs. C_{eff}) Effective concentration of drugs; T_{eff}) duration of action of drugs; ΔC) excess concentration of drugs.

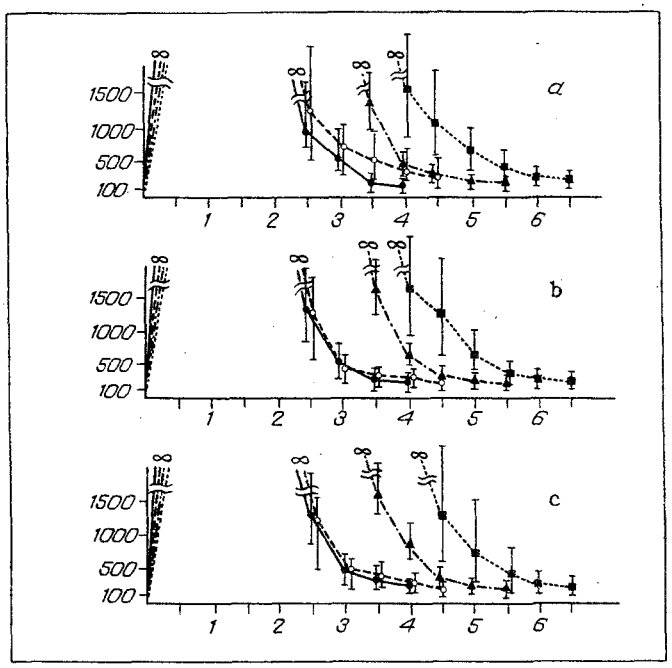

Fig. 4. Dynamics of changes in pain thresholds for respiratory (a), motor (b), and vocal (c) reactions to electrodermal stimulation of hind limb, during peridural anesthesia with amethocaine (1 ml of a 0.5% solution) in the pure form (filled circles), in sonicated egg phosphatidylcholine liposomes (empty circles), in unsonicated egg phosphatidylcholine liposomes (filled triangles), and in sonicated azolectin liposomes (filled squares). Abscissa, time (in h); ordinate, pain thresholds (in % of background). Ratio of phospholipids to amethocaine by weight 10:1.

To determine the efficacy of liposomal forms of local anesthetics, we studied the duration of peridural anesthesia with amethocaine in liposomes of different composition, prepared by different methods, in experiments on rabbits, and compared the toxicity of these forms with that of pure amethocaine injected intraperitoneally into mice.

TABLE 1. Comparison of Toxicity of Amethocaine and Its Liposomal Forms after Intraperitoneal Injection into Albino Mice

Serial No.	Experiment (amethocaine in liposomes)			Control (pure amethocaine)	
	composition	dose of amethocaine, mg/kg	mortality, %	dose of amethocaine, mg/kg	mortality, %
1.	Amethocaine 1%, egg phosphatidylcholine 5%	60	40	60	65
2.	Amethocaine 1%, azolectin 5%	60	0	60	65
3.	Amethocaine 1%, azolectin 5%	75	45	75	100

With peridural anesthesia by amethocaine (1 ml of a 0.5% solution) and its liposomal forms (phospholipids 5%), total blockade of pain sensitivity developed during the first 30 min after injection of the drug and it continued for 120-150 min, if amethocaine was used in the pure form and in single-layer egg phosphatidylcholine liposomes, 180-210 min if amethocaine was used in multilayered egg phosphatidylcholine liposomes, and 210-240 min if amethocaine was injected in single-layer azolectin liposomes (Fig. 4). Furthermore, after the end of the blockade, restoration of the pain thresholds to near-background values, in the case of anesthesia with liposomal forms of amethocaine (and in particular, azolectin liposomes), proceeded more slowly than with anesthesia by the pure preparation. These results are in good agreement with the theoretical suggestions given above and with the results of the NMR studies.

The investigation thus demonstrated that liposomal forms with low toxicity and with a long duration of action can be created. In our view the most promising of these liposomal forms are those of local anesthetics for long-term regional anesthesia.

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