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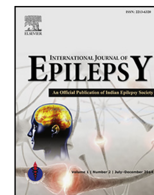

Research paper

Divergent interaction profiles of gabapentin and levetiracetam with dipalmitoylphosphatidylcholine lipids

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ABSTRACT

Background/Objectives: The lipid solubility of antiepileptic drugs directly affects central nervous system availability. In relation to this, the interactions of gabapentin and levetiracetam with dipalmitoylphosphatidylcholine lipids depending on concentrations were comparatively investigated in the present study.
Methods: The effects of gabapentin and levetricetam as a function of concentration (1–5–10–20 mol%) on biophysical parameters of dipalmitoylphosphatidylcholine multilammelar vesicles were studied by differential scanning calorimetry and fourier transform infrared spectroscopy.
Results: The data revealed that gabapentin at all concentrations and levetiracetam at 1–5 mol% lowered main transition temperature, enthalpy, cooperativity, lipid fluidity, lipid order, and increased hydrogen binding capacity of glycerol and phosphate groups. However, 10–20 mol% of levetiracetam tend to show different effect on transition temperature, which could also reflect its opposing effect on lipid order and glycerol and phosphate group's hydrations.
Conclusions: According to the corresponding findings depending on concentrations both drugs incorporate into phosphatidylcholines, perturbing the packing of lipids and affecting their thermotropic properties. Their binding affinity to acyl chains and hydrophilic parts of lipids was found to highly correlate with lipid-water partition and their solubility degree in water. Hence, the obtained results may offer evaluation of partition profile of the drugs into biological membranes depending on concentration.
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1. Introduction

Voltage-dependent sodium and calcium channel blockers and γ-aminobutyric acid (GABA) mimetics are approved as the most clinically useful groups of antiepileptic drugs (AEDs) to control the persistence of epileptic seizures. [1] Gabapentin (GBP) (Fig. 1A), marketed under brand name Neurontin, is one of the drugs belonging to this group. It is a structural analogue of the inhibitory neurotransmitter GABA. [2] Even though its action mechanism has not been fully clarified,[3] GBP has been shown to increase GABA levels in brain [4] and non-synaptic GABA neurotransmission. [5] In addition; it has potency to reduce voltage-dependent calcium [6] and sodium currents. [7] Another drug called levetiracetam (LEV) (Fig. 1B), trade name Keppra, is also with unresolved mechanism of action to suppress epileptic seizures. [8] However, evidence have shown that unlike traditional AEDs, the antiepileptic effect of LEV is based on inhibition of hypersynchronization of epileptiform burst firing, therefore producing an inhibition of the spread of seizure activity. [9] It is also considered to interact with a synaptic vesicle glycoprotein [10] and inhibit presynaptic calcium channels, hence reducing neurotransmitter release. [11] Additionally, its variable effects on synaptic transmission at hippocampal synapses have been reported. [11] Depending on clinical studies, both GBP and LEV are accepted as safe and well-tolerated with promising pharmacokinetic properties. [2,8]

Like many other drug molecules, for absorption into the bloodstream and distribution throughout the body both GBP and LEV must cross one or more phospholipid bilayers to reach their site of action and to elicit their response in the brain. Even if their targets are not the lipid bilayer but rather cytosolic proteins or bilayer-inserted proteins, bilayer insertion is prerequisite to acquire their correct orientations upon contacting with lipid bilayer and diffuse along these bilayers to meet their targets. [12] Thus, molecular interactions of such drugs with membrane lipids determine their orientation and conformation in membrane systems, and hence play an important role in transport,

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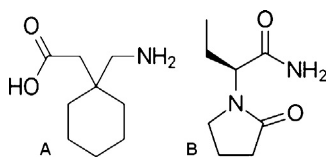

Fig. 1. Chemical structure of GBP (A) and LEV (B).

distribution, accumulation, and eventually their efficacy. [13,14] More importantly, they affect their central nervous system availability. Therefore, the affinity of these drugs to membrane can provide useful information for the understanding of their partition profile into biological membranes, and relatively effectiveness. This may further develop potent drugs for epilepsy treatment as suggested by others. [13]

Considering the solubility in water and low protein binding capacity of GBP and LEV they have higher propensity to interact with membrane lipids. This may also facilitate their rapid and ready crossing of blood brain barrier. [15,16] However; there is no published study yet available reporting how GBP and LEV partition into membrane lipids. On the other hand, since cell membrane consists of many different types of lipids, it is difficult to interpret interactions of drug with certain types of lipids with studies using whole cell membrane fraction. [17] Moreover, experiments using whole cells or natural cell membrane patches are often time and cost-intensive, and there is lack of suitable techniques for real time measurement. The use of artificial membrane systems is a general approach for such research. Relatively, with aiming to provide a general perspective about how GBP and LEV incorporate into membrane lipids their interactions with dipalmitoylphosphatidylcholine (DPPC) multilamellar vesicles (MLVs) were comparatively investigated in the present study; as earlier works performed for different kinds of molecules. [18–20] DPPC phospholipid bilayers used in the current work are considered suitable model systems to mimic cell membranes, and thus to study membrane structure and properties since phosphatidylcholines (PCs) are major lipid components of eukaryotic cell membrane. Additionally, their structural and thermodynamic properties are well-defined, which may be needed for the research of drug-lipid interactions. [18,20] Thus, the interactions of GBP and LEV with DPPC MLVs in terms of lipid phase behavior, order and dynamics and hydration state of the polar part of DPPC MLVs were examined using differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy, which have not been previously reported to the best of our knowledge.

2. Material and methods

2.1. Chemicals

Gabapentin [1-(Aminomethyl) cyclohexaneacetic acid], levetiracetam [(*S*)-2-(2-*O*xopyrrolidin-1-yl)butanamide], dipalmitoylphosphatidylcholine (DPPC) and phosphate buffered saline (PBS) tablets were purchased from Sigma (St. Louis, MO, USA). All chemicals were obtained from commercial sources at the highest grade of purity available.

2.2. Liposome preparation

By following the previous reports [18,20] DPPC MLVs in the absence and presence of GBP and LEV (1–5-10–20 mol%) were prepared. Briefly, 5 mg DPPC lipids were dissolved in chloroform and evaporated under nitrogen flow and desiccated under vacuum overnight to remove solvent. Then, thin films of dried lipids were obtained and re-suspended in phosphate buffer, pH 7.4. MLVs were formed by vigorous vortexing the mixture for 30 min at least 15 °C above main phase transition temperature of DPPC lipids. In order to produce drug containing MLVs, the required amount from stock solution was initially placed in tube. The excess of organic solvent was removed by a stream of nitrogen, DPPC in chloroform was added and MLVs was prepared as described above.

2.3. FT-IR studies and analysis

20 μL of liposomes with and without drugs, and buffer placed between calcium florur (CaF_2) windows with 12 μm sample thickness were scanned using a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer, Inc., Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. The experimentation were performed at 25–60 °C to investigate if the drugs behave differently in the gel and the liquid crystalline phase of DPPC MLVs. Before the scans the samples were incubated for 5 min. The interferograms were averaged for 100 scans at 2 cm^{-1} resolution. All experiments were three times performed. The sample compartment in the FT-IR spectrometer was continuously purged with dry air to prevent water vapor. The spectrum of air was automatically subtracted by Perkin Elmer Spectrum One software.

To improve resolution of the infrared bands of interest, buffer spectrum at corresponding temperature was subtracted to eliminate overlapping effect of the OH stretching modes (3400–3200 cm^{-1} and 1800–1500 cm^{-1}) from water molecules in buffer. The subtraction process was performed till bulk water region located around 2100 cm^{-1} was flattened using same program.

Perkin Elmer Spectrum One software was used to determine variations in peak positions and bandwidths, as well. The band positions were measured from the center of weight (0.80 × peak height positions) and bandwidth values were calculated as the width at 0.75 × height of the signal in terms of cm^{-1}. For visual demonstration of the spectral differences in the spectra, the spectra were normalized with respect to the specific bands. Data analysis was performed on water subtracted sample spectra.

2.4. DSC studies

For DSC studies, MLVs were prepared by following the procedure in FT-IR sample preparation mentioned above. 50 μL MLVs suspensions were encapsulated in hermetically sealed standard aluminum DSC pans. An indium containing pan was used as reference during the analysis. Measurements were performed using a Shimadzu DSC-60 Calorimeter (Shimadzu Corporation, Tokyo, Japan) equipped ascending and descending temperature mode operations. Scans were made at 0.5 °C/min. Only heating curves are presented. Samples were scanned three times to ensure the reproducibility of the endotherms and to eliminate the thermal history of the sample. Data were analyzed using TA 60 software provided by Shimadzu. The temperature at the peak maximum was defined as the transition temperature. The enthalpy (ΔH_{cal}) values were calculated by integrating the peak area under main transition. Cooperativity unit (CU), which is a measure of the mean number of lipid molecules undergoing transitions, was calculated as in Turker et al.. [18]

2.5. Statistical analysis

The mean of at least three experiments was plotted and calculated together with the standard error of mean in the figures and tables. Statistical significance was assessed using Mann-Whitney nonparametric test. Significant differences were statistically considered at the level of $p \leq 0.05$.

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