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Interaction of different statins with model membranes by NMR data

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ABSTRACT

Hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins reduce the amount of low-density lipoprotein (LDL) cholesterol, which is known as a well-established risk factor for atherosclerosis. Despite the fact that statins have a common pharmacologic target essential to sterol biosynthesis, their efficacy, safety, and potential non-LDL actions vary significantly for different statins. There is a hypothesis that pharmacological features of statins depend on their location in cell membrane, but to the present day there is a lack of information in literature on interactions of statins with the surface of the cell membrane in liquid media. The results of NMR experiments showed that all studied statins form intermolecular complexes with models of cell membranes (dodecylphosphocholine micelles) in water solution. Locations of pravastatin, simvastatin, fluvastatin and cerivastatin on model membranes were established by NOESY NMR data. Distinctions in their positions can explain differences in pharmacological properties of studied compounds.

1. Introduction

Hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins reduce the amount of low-density lipoprotein (LDL) cholesterol, which is known as a well-established risk factor for atherosclerosis. Despite the fact that statins have a common pharmacologic target, which is essential to sterol biosynthesis, their efficiency, safety, and potential non-LDL actions differ considerably depending on their chemical structure. These drugs vary significantly in their rate of absorption, amount of protein binding, degree of renal excretion, metabolism, hydrophilicity, interaction with other drugs, and potency on a per-weight basis [1–5]. Origins of these differences are not well investigated. There is a hypothesis that metabolism and safety of statins depend on their location in the cell membrane [6–8], but to the present day there is a lack of information in literature on interactions of statins with the surface of the cell membrane in liquid media. Therefore, investigation of interaction of statins with cell membrane in solution can shed light on the reasons of their pharmacologic differences.

Nuclear Overhauser Effect NMR spectroscopy (NOESY) is an effective method for studying intermolecular interactions, particularly including different medicines [9–13]. It can provide information on the structure of molecular complex and on certain fragments of the molecule which are responsible for the binding. However, the capabilities of modern NOESY NMR spectroscopy in the field of cell study are still very limited. There is a problem in using this technique for investigation of molecular interactions in phospholipid membranes because T_2 proton relaxation times of phospholipid aggregates are too short relative to the NMR chemical-shift timescale [14–15]. Nevertheless, interactions of different drugs with cell surface can be effectively studied by NMR using model membranes such as dodecylphosphocholine micelles. Zwitterionic dodecylphosphocholine (DPC) are one of the most widely used surfactants for cell membrane modeling in the field of NMR structural biology [16–20]. DPC micelles effectively mimic eukaryotic membranes, because DPC has exactly the same head group as phosphatidylcholines that are predominant class in eukaryotes.

The aim of this work is to study the interaction of statins (pravastatin, simvastatin, fluvastatin, and cerivastatin) having different pharmacological properties [1–5] with models of cell membranes (DPC micelles) by NMR spectroscopy. This paper is an attempt to explain the pharmacological differences of statins in terms of distinctions in their location on the cell membrane.

2. Material and methods

2.1. Samples preparation

All statins and dodecylphosphocholine were purchased from Sigma-Aldrich Rus (Moscow, Russia) and used without further purification. Pravastatin, fluvastatin, and cerivastatin were dissolved in D_2O and D_2O + DPC with concentration of 7.1, 7.3 and 6.5 mM respectively. Simvastatin was dissolved in D_2O + DPC with concentration of 3.1 mM. Solutions containing micelles were prepared using combination of deuterated (>98%) and undeuterated DPC. The concentration of DPC in D_2O solution was greater than the critical micelle concentration and was equal to 7.7 mM for non-deuterated DPC and 17.5 mM for deuterated DPC. Proper amounts of DPC have been weighted to prepare stock water solutions. After surfactant solubilization in D_2O, the stock

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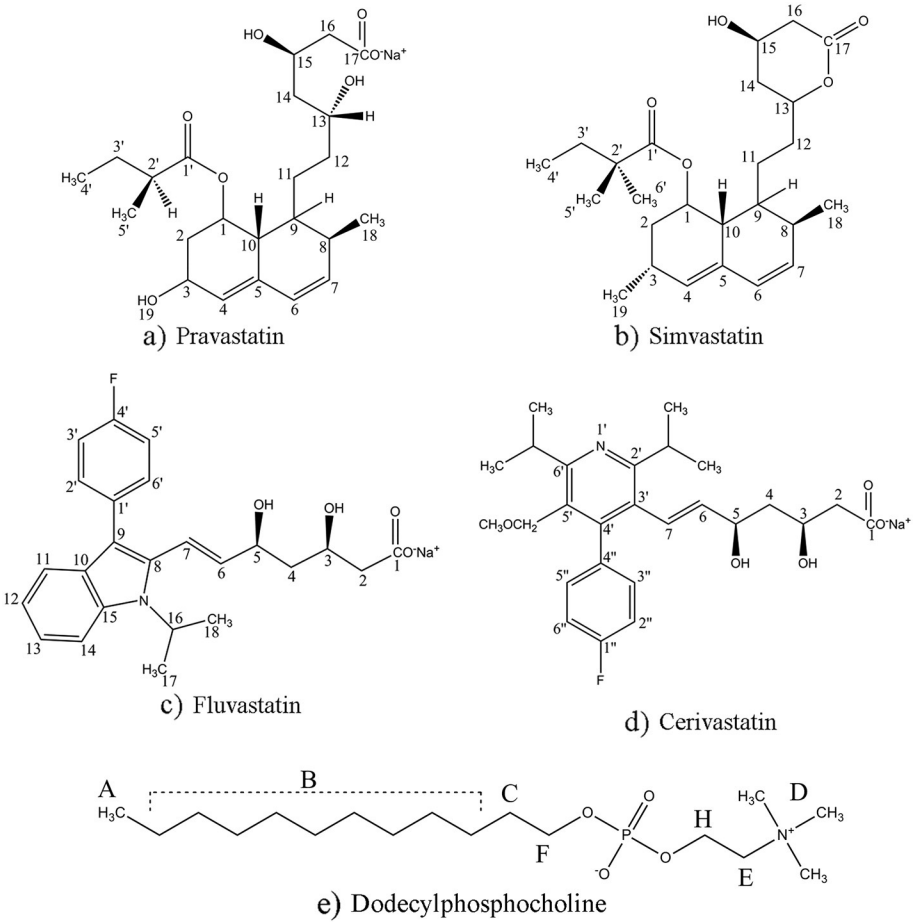

Fig. 1. Chemical structures of studied statins (a–d) and dodecylphosphocholine molecule (e).

solutions have been vortexed and then sonicated in an ultrasound bath for 5 min. After stock solution preparation, proper amounts of statins were added, sample have been vortexed and then left to equilibrate overnight at the room temperature.

2.2. NMR spectroscopy

All NMR experiments were performed on a Bruker Avance II 500 NMR spectrometer equipped by a 5 mm probe using standard Bruker TOPSPIN software. Temperature control was performed using a Bruker variable temperature unit (BVT-3000) in combination with a Bruker cooling unit (BCU-05). Experiments were performed at 303 K without sample spinning. Chemical shifts are given in values of ppm, referenced to residual solvent signals (4.72 ppm for ^{1}H in D_2O). ^{1}H NMR data were collected with 32 k complex data points.

Assignments of ^{1}H and ^{13}C NMR signals of compounds were achieved from signal multiplicities, integral values and characteristic chemical shifts from the through-bond correlations in 2D COSY spectra, through-space correlations in 2D NOESY spectra as well as from ^{1}H—^{13}C heteronuclear correlations in 2D HSQC and HMBC spectra.

All two-dimensional experiments were performed with 2 k × 512 data points; the number of transients (96 scans) and the sweep widths were optimized individually. In the homonuclear ^{1}H—^{1}H COSY (Bruker pulse program cosygpqf) and 2D ge-NOESY [21] experiments were performed with the pulsed filtered gradient technique [22], the relaxation delay was set to 2 s, and the 90° pulse length was 7.5 μs. Mixing time values were 0.5, 0.4, 0.3, 0.2, and 0.1 s. The resulting FIDs were zero-filled to a 2k × 1k data matrix and apodized with a sine function for COSY and a shifted sine function for NOESY in both the ω1 and ω2 dimensions prior to Fourier transformation. Heteronuclear spectra were recorded with 2k × 512 data points, zero-filled in F1 to a 2k × 512 data matrix, and apodized in both dimensions with a shifted sine function. HSQC experiments (hsqcetgpsp) were acquired using adiabatic pulses for inversion of ^{13}C and GARP-sequence for broadband ^{13}C-decoupling, optimized for ^1J(CH) = 135 Hz. ^{1}H—^{13}C long-range spectra HMBC (hmbcgplpndqf) were performed with ^nJ(CH) set to 7 Hz. 1D ROESY experiments were carried out using pulse sequences, described

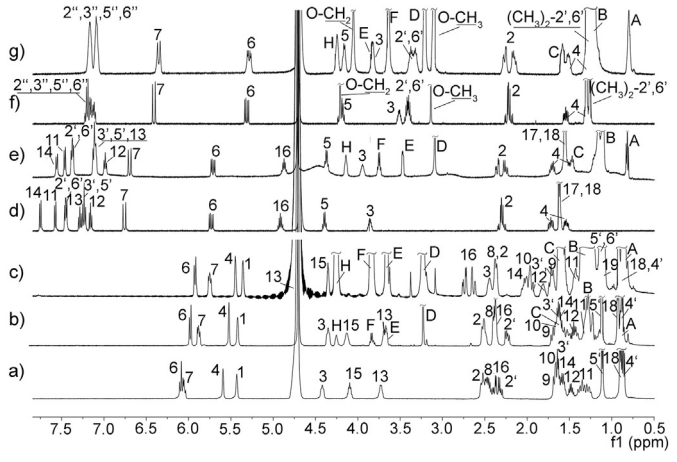

Fig. 2. ^{1}H NMR spectra of (a) pravastatin, (b) pravastatin + DPC micelles, (c) simvastatin + DPC micelles, (d), fluvastatin, (e) fluvastatin + DPC micelles, (f) cerivastatin, (g) cerivastatin + DPC micelles in D_2O solution, T = 303 K. Figures correspond to the numeration of statin atoms. Latin letters correspond to DPC atoms (see Fig. 1). Unlabeled signals correspond to the signal of H_2O, satellites and minor impurities.