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Araştırma Makalesi

A Calorimetric Investigation of the Effects of Acipimox on DPPC Model Membranes

Nazli EZER OZER[1], Ipek SAHIN[2]*

[1]Altinbas University, Faculty of Medicine, Department of Biophysics, Istanbul
[2]Ege University, Faculty of Science, Department of Physics, Izmir

*Sorumlu Yazar: ipek.sahin@ege.edu.tr

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ÖZ

Model membranlar, biyolojik membranları taklit eden temel sistemler olarak yaygın şekilde kullanılmaktadır. Bu çalışmada, dipalmitoil fosfatidilkolin (DPPC) ile lipit düşürücü ilaç asipimoks arasındaki fiziksel etkileşim, farklı asipimoks konsantrasyonları ve sıcaklığa bağlı olarak Diferansiyel Tarama Kalorimetri (DSC) tekniği kullanılarak araştırıldı. DPPC içeren model membranların faz geçiş sıcaklığı, faz geçiş entalpisi ve faz geçiş eğrisi yarı yükseklik genişliğindeki değişimler değerlendirildi. DSC sonuçlarına göre saf DPPC model membranlara asipimoks ilavesi ile 41 °C civarındaki ana faz geçiş sıcaklık eğrisi biraz daha yüksek sıcaklıklara kayarken, 35 °C civarındaki geçiş öncesi sıcaklık eğrisi kaybolmadı. Ayrıca, artan asipimoks konsantrasyonları, model membran DPPC'nin DSC termogramlarında hafif bir genişlemeye neden oldu. İlaçların biyomembranlardaki farmakolojik aktivitelerinin anlaşılması onların hücre içi aktivileri açısından çok önemli olduğu için, lipit düşürücü ilaç asipimoks ile lipitler arasındaki etkileşimin araştırılması, asipimoksun moleküler düzeydeki biyolojik etkilerine katkı sağlayabilir.

Anahtar kelimeler: Asipimoks, Model Membranlar, DPPC, DSC, İlaç-Membran Etkileşimi

Asipimoksun DPPC Model Membranlar Üzerindeki Etkilerinin Kalorimetrik İncelenmesi

ABSTRACT

Model membranes are widely used as basic systems which mimic biological membranes. In this study, the physical interaction between dipalmitoyl phosphatidylcholine (DPPC) and the lipid-lowering drug acipimox was investigated using Differential Scanning Calorimetry (DSC) technique depending on different acipimox concentrations and temperature. The changes in the phase transition temperature, phase transition enthalpy and phase transition curve half-height width of the DPPC containing model membranes were taken into account. According to the DSC results, with the addition of acipimox into pure DPPC model membranes, the main phase transition temperature curve, which is around 41 °C, shifted to slightly higher temperatures, while the pre-transition temperature curve, which is around 35 °C, did not disappear. Moreover, increasing acipimox concentrations caused a slight broadening of the DSC thermograms of the model membrane of DPPC. Acipimox faintly changed DPPC's structural characteristics, notably when introduced to the bilayer at high concentrations. DPPC and acipimox may not have enough chemical bonding to explain this interaction. Furthermore, acipimox may be found in the hydrophilic part of the DPPC. Since it is very important to understand the pharmacological activity of drugs in biomembranes, the investigation of the interaction between lipid-lowering drug acipimox and lipids may contribute to the biological effects of acipimox at the molecular level.

Key words: Acipimox, Model Membranes, DPPC, DSC, Drug-Membrane Interaction

INTRODUCTION

Acipimox (5-methylpyrazine carboxylic acid-4-oxide) (Figure 1) is a lipid-lowering agent and an analog of niacin. It works by inhibiting lipolysis in adipose tissue, which blocks the synthesis of VLDL (very-low-density lipoprotein) and LDL (low-density lipoprotein) cholesterol (Minigh, 2007; Christie et al., 1996). Despite its role in the treatment of disorders related to high levels of fat in blood such as hyperlipidemia, the precise mechanism underlying acipimox action remains unclear.

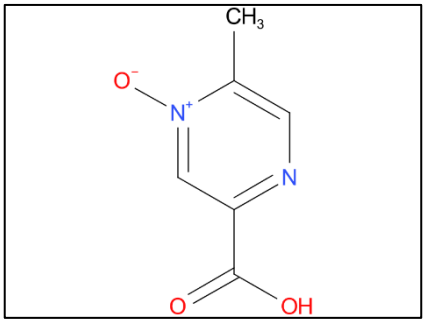

Figure 1. Chemical structure of acipimox

The cell membranes (plasma membranes) are important cellular components with wide variety of roles (Severcan and Dorohoi, 2008). They protect the intracellular environment from the extracellular environment, control the diffusion of ions and molecules, and act as a supporting matrix for the cell (Li et al., 2018). Plasma membranes have an extraordinarily complex structure containing different types and proportions of lipids, proteins, and carbohydrates (Watson, 2015). This complex structure can create difficulties in interpreting the results in the study of agent-membrane or drug-membrane interactions (Severcan et al., 2005). In order to help understand such interactions in these systems, it is a general approach to start studies with simpler structured model membranes whose components can be changed in a controlled manner (Ergun et al., 2014). Liposomes, kinds of model membranes, consist of a lipid bilayer and have a spherical structure. Different types of liposomes can be obtained by varying the surface charge, lipid composition, size, and preparation technique (Akbarzadeh et al., 2013). Multilamellar liposomes (MLVs) include a large population of vesicles between 100 and 1000 nm, and they are highly preferred in biophysical and pharmacological studies. The reason why they are widely used is that MLV can be manufactured using a wide range of lipid compositions and is effective for encasing a number of compounds. They are the easiest to make of all the liposome preparations, and they can be used in many drug release tests to get a more uniform size distribution of liposomes. SUV (small unilamellar vesicles) and LUV (large unilamellar vesicles) can be obtained if desired. Moreover, they are suitable for cosedimentation, coflotation, and solid-state NMR investigations (Lombardo and Kiselev, 2022; Szoka, Jr. and Papahadjopoulos, 1980; Zhao and Lappalainen, 2012).

The main components of biological and model membranes are phospholipids. Examples of these phospholipids are dipalmitoyl phosphatidylcholine (DPPC), dipalmitoyl phosphatidylglycerol (DPPG), or cholesterol. Phospholipid bilayers have hydrophilic heads in contact with water and hydrophobic tails directed towards the middle of the bilayer (Severcan and Dorohoi, 2008). Phospholipids used in model membrane formation can be neutral or charged lipids with different acyl chain lengths. As known, chain length and unsaturation in the phospholipid tails affect lipid phase transitions and membrane fluidity, while a difference in the phospholipid head group leads to different packaging patterns and different intermolecular interactions (Frallicciardi et al., 2022). Phospholipids have the ability to form more than one solid structure. This behavior represents interconversions between phases, forming various subgel, gel, or liquid crystalline phases as a function of temperature, water content, and composition (Koynova and Tenchov, 2013). L_c is a subgel phase in which the alkyl chains are all-trans configuration, and the head groups are also highly ordered. The L_β (for phosphatidylethanolamines) or L_β' (for phosphatidylcholines) phase is called the gel phase. In the gel phase, the head group becomes disordered, while the alkyl chains remain in the all-trans configuration. As the temperature increases, a transition to the P_β' phase, called the ripple-gel phase, occurs for phosphocholine and some other phospholipids. This transition is called the pre-transition because it is followed immediately by the L_α liquid-crystalline phase. The melting of the lipid bilayer from P_β' to $L\alpha$ phase called as the main phase transition (Figure 2). The sign (') in the phase symbols denotes tilted alkyl chains in phases (Alakoskela, 2005; Kranenburg and Smit, 2005). Each phospholipid has a unique pre-transition temperature (T_p) and main phase transition temperature (T_m). The permeability of lipid bilayers increases when they change from a regular gel

phase to an irregular liquid phase. Permeability is an important physical property of liposomes as it affects the movement of agents across the membrane (Chen et al., 2018).

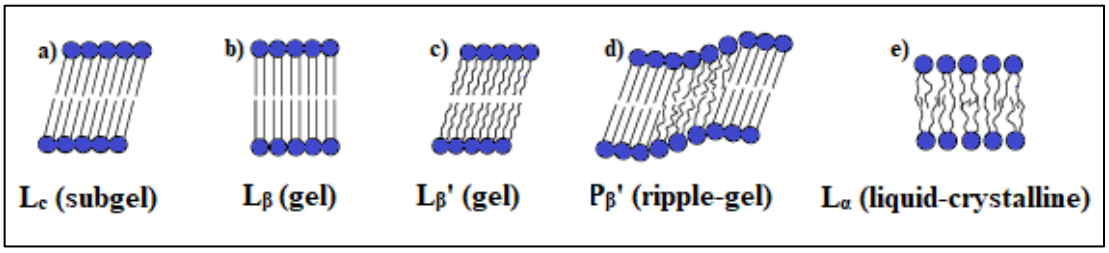

Figure 2. Schematic representation of the different phases of phospholipid bilayers (Kranenburg and Smit, 2005).

Model membranes are widely used to study drug-membrane interactions. Various drugs may behave differently at low and high concentrations. Drugs can change order/disorder state and fluidity of the membrane interacting with hydrophobic and hydrophilic parts. Differential Scanning Calorimetry (DSC) technique has been frequently used in model membrane studies to determine the thermal properties of biological systems, since model membrane studies depending on drug concentration and thermal changes of the effects of agents on membranes are especially important (Ceckler and Cunningham, 1997; Sariisik et al., 2019; Sahin et al., 2013). In model membrane studies, the DSC technique has been used to directly monitor the phase transition behavior (Cakmak Arslan and Severcan, 2019).

Although there are many studies on the clinical use of acipimox in the literature (Sirtori et al., 1981; Taskinen and Nikkilä, 1988; Worm et al., 1994; Saloranta et al., 1993; Barayan et al., 2020; Vardarli et al., 2022; Lupachyk et al., 2012; Vestergaard et al., 2017; Kaur et al., 2020), there is no studies examining its physical interaction with model membranes at the molecular level. Therefore, in this study, we aimed to use DSC to investigate the physical interactions between acipimox and DPPC MLVs by monitoring the phase transition behavior. Molecular interactions of the lipid-lowering drug with model membranes containing mainly phospholipids may assist future studies by helping to better understand the action mechanism of acipimox.

MATERIALS AND METHOD

Chemicals

Acipimox, DPPC and phosphate buffered saline (PBS) tablets were bought from Sigma (St. Louis, MO, USA). All chemicals were procured from commercial sources at the highest purity available.

DSC Studies

For obtaining DSC thermograms, pure DDPC liposomes were prepared in the absence and the presence of different concentrations of (1 mol %, 3 mol %, 9 mol %, 12 mol %, 15 mol %, 18 mol %) acipimox, according to procedure which is reported by Severcan et al. (2005). In order to obtain DPPC liposomes in which different acipimox concentration containing, stock solution was prepared via dissolving acipimox in ethanol first. The necessary amounts of acipimox concentrations were put in eppendorfs and evaporated with the help of nitrogen flux. After that, 2 mg of DPPC was dissolved by using chloroform. Dried lipid films were obtained by removing chloroform with nitrogen and pumping it for at least 3 h under vacuum by means of Christ LT-105 spin vac. For hydration, 50 µl PBS buffer solutions, pH 7.4, were added to dry lipid films. MLVs were prepared by vortexing the samples at a temperature above the gel-to-fluid phase transition of DPPC (around 41 °C) for 20 min. 50 µl liposome suspensions were encapsulated in standard aluminum DSC pans. Experiments were performed with TA Q 2000 DSC device with a heating rate of 1 °C/min. Calorimetric data was obtained in a temperature range of 25-60 °C. All measurements were taken three times and the same results were observed at each repeat. The enthalpy (ΔH J/g) values were carried out as a result of the calculation of the area under main transition.

RESULTS AND DISCUSSION

The biological activity of drugs can change the physical properties of lipids, for instance the thermotropic phase transition profile. The variation of the phase transition profile of acipimox on model membranes may be critical for understanding the drug's mechanism of action and predicting drug-membrane interactions. During drug-membrane interaction, any change in phospholipid phase state is believed to affect membrane function by modifying protein and lipid structure. Moreover, physical examination of model

membranes with concentration-dependent drug interactions is quite interesting since some drugs act differently at various concentrations. It is also important to find effective usage of drugs in treatment (Severcan et al., 2005; Sariisik et al., 2019; Ezer et al., 2017).

The most important technique that measures the thermal properties of samples by correlating them with their temperature and physical properties is called calorimetry. It also has the ability to directly determine the enthalpy of the system. DSC, one of the most popular calorimetric techniques, has been commonly used in the temperature and concentration dependent model membrane studies, since one can monitor changes in phase transitions and structural changes in lipid bilayers and drug delivery systems. Moreover, the position, sharpness, and shape of the DSC thermograms shows the change in conformation of the molecule of interest. Thermodynamic data obtained by adding different concentrations of acipimox into pure DPPC liposomes helped us to interpret how the drug might affect the stability and physical properties of the membrane as reported previously by Severcan and Dorohoi, 2008; Severcan et al., 2005; Ergun et al., 2014; Ezer et al., 2017.
Figure 3 shows the DSC curves of DPPC liposomes in the absence and presence of increasing concentrations of acipimox. Pure DPPC liposomes has a pre-transition peak at 35 ^{o}C and a main transition peak at 41 ^{o}C. Addition of the acipimox into the DPPC, the pre-transition peak was slightly broadened suggesting that there may be a little perturbation of the ripple phase. However, since different molecules are localized in the polar region of phospholipids, the broadening of the pre-transition peak cannot be referred to special molecular changes as reported before (Ezer et al., 2017; Turker et al., 2011).

As seen from Figure 3 and Figure 4a, addition of acipimox into pure DPPC MLVs decreased the intensity of the main phase transition curve, and a slight shift to higher values of T_m was observed as the concentration of acipimox increased. Since the shift of the T_m is related with the hydrocarbon chains of the lipids, it provides knowledge about the order/disorder state of the phospholipid bilayer. If the phase transition temperature shifts to lower values with the addition of low and high concentrations of the agent, cooperation between the acyl chains of lipid molecules and van der Waals interactions are reduced, the number of gauche conformers and order of the system decreases; if the phase transition temperature of the phospholipid shifts to higher values, the agent increases the cooperation between the lipid acyl chains, resulting in an increase in the order of the system (Severcan et al., 2005; Sariisik et al., 2019; Ezer et al., 2017). As Tm slightly shifted to higher temperatures, means that the change of all trans conformers to gauche conformers occurs at higher temperatures and acipimox made the membrane more stable ((Severcan et al., 2005; Sariisik et al., 2019; Toyran and Severcan, 2002; Toyran and Severcan, 2003; Severcan et al., 2000). The broadening of the DSC peaks suggests that the cooperation between the lipid chains is reduced. Therefore, current findings may indicate that cooperativity between acyl chains of DPPC liposomes and acipimox slightly decreased since a narrower peak means higher cooperativity of hydrocarbon chains. Moreover, the slightly broadening in the phase transition peaks ($\Delta T_{1/2}$) at low and high concentrations means that acipimox may not enter the (C_2-C_8) region of the hydrocarbon chains and were probably localized at the interface of the DPPC bilayer (Figure 4b) (Severcan et al., 2005; Turker et al., 2011).

The heat content of a system is called enthalpy (H). The change of enthalpy (ΔH) for a reaction is approximately equivalent to the lost or gained energy amount during the reaction. At constant pressure, when external energy is supplied to the system, the enthalpy of the system increases, and when the system gives heat to the environment, the enthalpy of the system decreases. In endothermic reactions, the system gains energy and ΔH is positive (ΔH > 0). Drugs or other agents exert their effects by making chemical bonds or breaking these bonds with the acyl chains or head groups of membrane lipids according to their molecular properties and concentrations. These interactions cause changes in the T_m and ΔH values of the DSC curves. The decrease in T_m and ΔH values indicates that the agents make strong hydrophobic bonds with the membrane. Moreover, it can be explained that the cooperativity and van der Waals interactions between lipid molecules decrease (Yagofarov et al., 2018; Yagofarov et al., 2022; Wu and Yalkowsky, 2009; Sanghvi and Yalkowsky, 2006). Hovewer, as observed from Figure 4c, ΔH value increased with the addition of acipimox at all concentrations into DPPC MLVs in our study. Since the enthalpy change gives information about the structure of the system, this result can show that bond-breaking costs more energy than what is provided in bond-making in between acipimox and DPPC liposome interaction. Any structural change in the molecular packaging of the lipid acyl chain in the presence of the drug affects the enthalpy change. Therefore, an increase in both T_m and ΔH reflects the stability of the phospholipid bilayer and as mentioned above, the agent can be said to interact with the relatively hydrophilic portions of the lipids (Sariisik et al., 2019; Yagofarov et al., 2018; Yagofarov et al., 2022; Wu and Yalkowsky, 2009; Sanghvi and Yalkowsky, 2006).

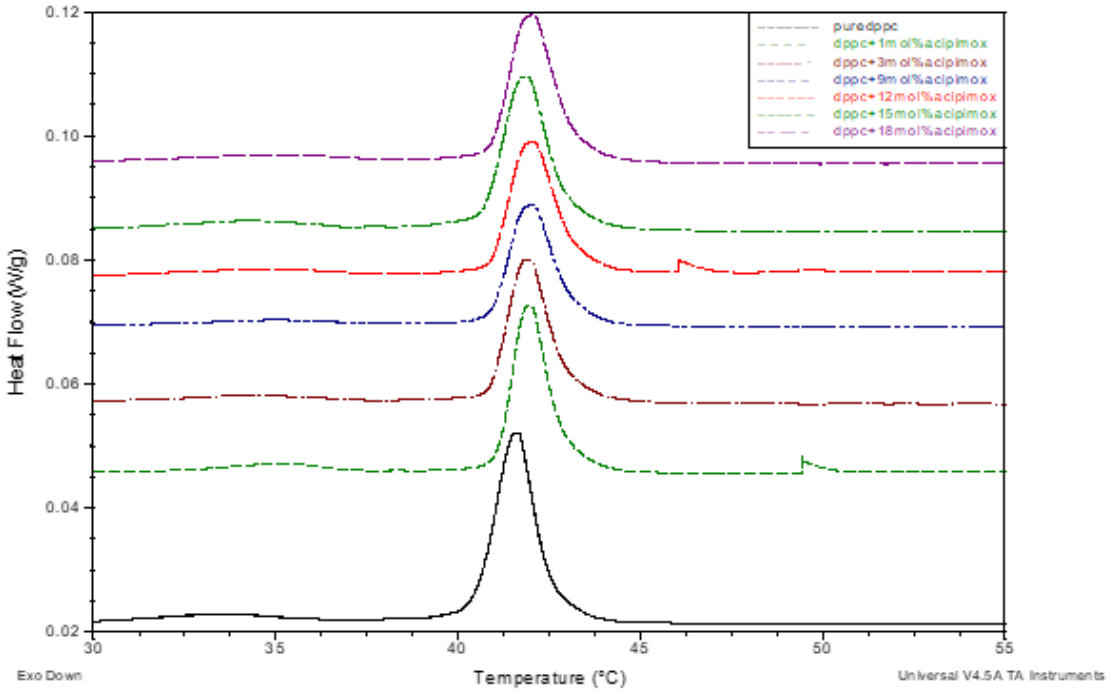

Figure 3. The DSC thermograms for DPPC liposomes in the absence and presence of low and high concentrations of acipimox.

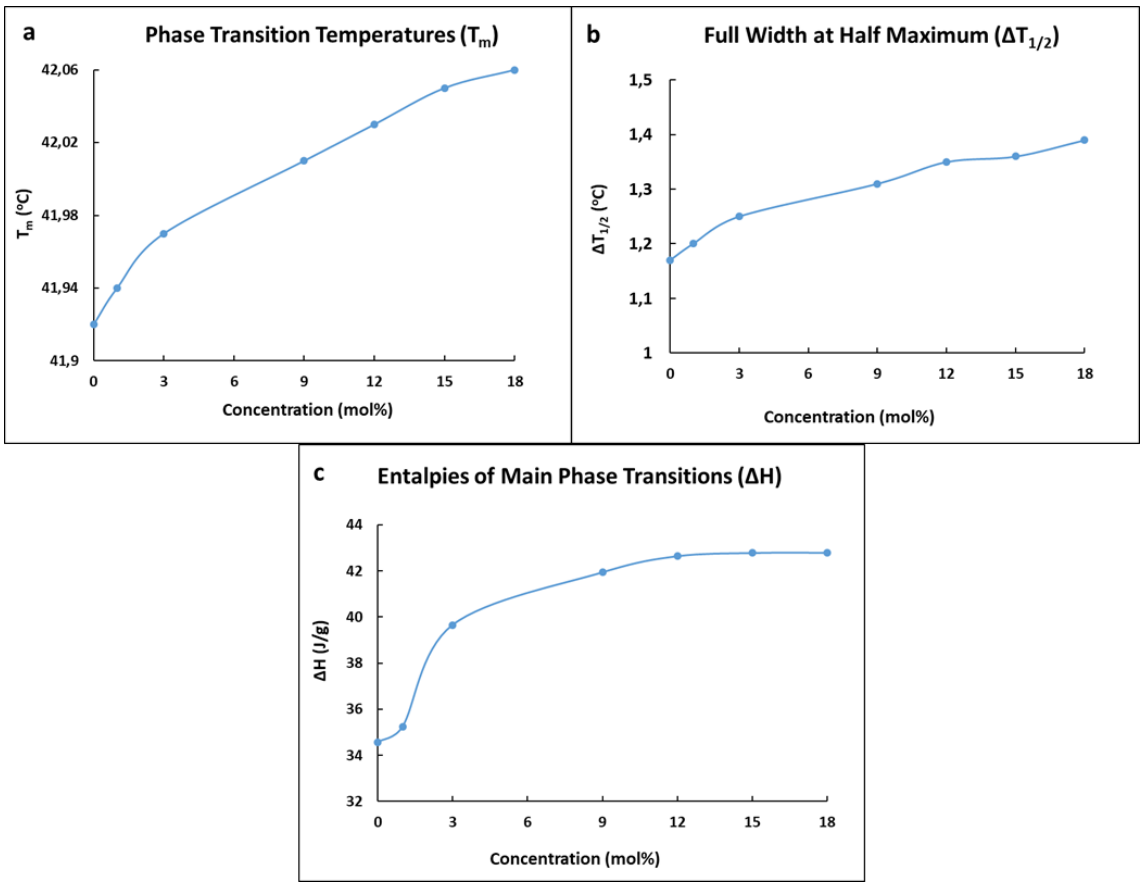

Figure 4. (a) Main phase transition temperatures (T_m), (b) full width at half maximum ($\Delta T_{1/2}$) and (c) enthalpies of the main phase transitions (ΔH) of DPPC liposomes obtained by the addition of acipimox.

Such obtained data from the present study might be related to the cellular action of acipimox on membrane lipids. It is known that integral membrane proteins interact with fatty acyl chains of membrane lipids through hydrophobic matching. In contrast, peripheral membrane proteins are predominantly bound to lipid head groups by electrostatic and hydrogen-bond interactions (Brasseur et al., 1997). Relatively, we found that acipimox has ability to interact with hydrophilic portions of lipids, therefore; such ability of acipimox on lipids might further indirectly affect the conformation of peripheral proteins and/or polar part of integral proteins. Considering that although there are no studies in the literature examining the interaction of acipimox with membranes or membrane phospholipids at molecular level by any technique, clinical studies are available. Moreover, the therapeutic effect of acipimox on various diseases has been reported widely. In a study investigating the hypolipidemic effect of acipimox in two double-blind crossover trials versus placebo, Sirtori et al. (1981) observed a reduction in triglyceridemia and an increase in high-density lipoprotein cholesterol levels after treatment with acipimox as a novel lipolysis inhibitor. In another study investigating the different effects of acipimox (acute and chronic) therapy on glucose and lipid metabolism in type II diabetes patients, Saloranta et al. (1993) emphasized that administered acipimox acutely inhibited the appearance of non-esterified fatty acids (NEFA), which was associated with improved glucose uptake, but caused a marked increase in fasting NEFA concentrations after 4 weeks of treatment. Taşkınen et al. (1988) showed that acipimox given at a relatively low and well-tolerated dose (750 mg daily) to patients with hypertriglyceridemia was effective, but these results were not significant in short-term application. They also reported that acipimox was certainly less effective in severely hyper triglyceridemic patients, but its prolonged administration may result in changes in HDL levels and lower fraction distribution, most likely by lowering hepatic lipase. In another study examining the effects of high-dose acipimox as adjunctive lipid-lowering drug in familial hypercholesterolemia, Stuyt et al. (1998) found that when acipimox (750 mg/day) were added to therapy with simvastatin, total serum cholesterol (9%), LDL-cholesterol (9%), and serum triglycerides (21%) were significantly reduced. However, higher doses did not have a greater hypolipidemic effect. A significant reduction in apolipoprotein (apo)-B (11%) was observed, consistent with the decrease in serum cholesterol and LDL-cholesterol while HDL-cholesterol, apo-A1 and lipoprotein(a) were not change. Vickers et al. (2006) reported beneficial metabolic efficacy of combination therapy with acipimox enhanced the effect of growth hormone treatment on linear body growth in rats. Salvador et al. (2018) reported that combination therapy with omega-3 fatty acids and acipimox may offer an option to previously reported lipid-lowering treatments without serious opposite reactions. As mentioned above, the absence of a study in the literature examining the interaction of acipimox with membranes has made this study important.

SUGGESTIONS AND CONCLUSION

In conclusion, we used DSC technique to obtain information about the thermotropic properties of interaction between acipimox and DPPC membrane in our study. To gain a deeper perspective, we examined the possible interaction of niacin analogue acipimox with DPPC model membranes by studying its effect on thermal phase behavior and thermal changes. Our results suggested that acipimox slightly altered the structural properties of DPPC, especially when high concentrations of acipimox were added to the bilayer. One possible explanation for this interaction is that there may be not enough strong chemical bonding between DPPC and acipimox. Moreover, acipimox is a member of the pyrazine carboxylic acid class of chemical compounds. These are heterocyclic compounds with pyrazine rings that have been substituted with one or more carboxylic acid groups. They are polar molecules because they are both hydrogen bond acceptors (carbonyl -C=O) and donors (hydroxyl -OH). Since the hydrophobic center of the phospholipid membrane produces a practically impermeable transport barrier for most polar particles, acipimox may have only interacted with the hydrophilic surfaces of the DPPC membranes.

Such studies at the molecular level and physical examination of the membrane interactions of drugs used in the treatment of diseases such as acipimox may offer promising approaches to understanding the action mechanism of agents.

Conflict of Interest Declaration: : The authors have no conflict of interest concerned to this work.

Contribution Rate Statement Summary: The authors declare that they have contributed equally to the article.

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