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Effects of sulindac and naproxen on model membrane dynamics

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent non-specific inhibitors of the cycloxygenase pathway of inflammation, and therefore an understanding of the interaction process of these type of drugs with membrane phospholipids is of high importance. There are still many unresolved points about the exact molecular mechanism behind the action of NSAIDs. The present study was designed to investigate the effects of two NSAIDs, namely sulindac and naproxen, on phospholipid membranes. The effects of sulindac and naproxen at various concentrations (1, 6, 9, 12, and 18 mol%) on phospholipid model membranes prepared from dipalmitoylphoshatidylcholine (DPPC)-Cholesterol has been studied by turbidity technique as a function of temperature (25 ºC – 65 ºC; with 2 ºC intervals). Sulindac decreases the fluidity of the DPPC-cholesterol model membrane system at all temperatures studied, while naproxen increases the fluidity of the same model membrane system at low temperatures (below 40 ºC). Sulindac and naproxen alter the physicochemical properties of phospholipid membranes by changing the dynamics of the model membranes.

KEYWORDS

Nonsteroidal anti-inflammatory drugs (NSAIDs);
Sulindac;
Naproxen;
Model membranes;
Fluidity.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications today because of their efficacy in the treatment of pain, inflammation, and fever. Although their exact molecular mechanism of action is still not clear, inhibition of prostaglandin synthesis contributes significantly to their analgesic, anti-inflammatory, and antipyretic effects[1]. Recently some of the NSAIDs, especially sulindac, have emerged as a new cancer chemotherapeutic and chemopreventive therapy[1-3].

Although NSAIDs are classified into several subgroups based on their chemical structure[4], the common mechanism of action of all them is the inhibition of the cyclooxygenase (COX) enzyme. The COX pathway converts arachidonic acid into prostaglandins and thromboxans, contributing to several physiological functions[5]. It is well known that there are at least two COX isoenzymes, called COX-1 and -2, possessing distinct enzymatic activities[6]. Most of the NSAIDs are non-selective inhibitors of COX-2 due to the high degree of structural similarity[7]. Most serious side-effects are gastrointestinal damage including ulceration and haemorrhage, renal toxicity, inhibition of the platelet aggregation[8]. Additionally, studies have shown

alternative COX independent mechanisms of action inducing local ulcerations in the gastrointestinal tract[9]. These findings suggest the possibility of a direct membrane activity.

Although sulindac and naproxen are among the most widely investigated drugs in terms of their pharmacological action, less is known about their real effects on cell membranes[10]. The number of studies concerning the effect of these drugs, specifically on the membrane dynamics, are quite limited and contradictory. In a previous study, it was reported that sulindac alters the physical state of the membrane and strongly decreases fluidity of cellular membranes[11]. Several other groups also suggested that NSAIDs strongly reduce membrane fluidity[9,12]. On the other hand, Hwang and Shen (1981) reported in a differential scanning calorimetry (DSC) study that sulindac increases the fluidity of the membrane systems. In their study, the interaction of the active sulfide metabolite of sulindac with phosphatidylcholine (PC) liposomes was investigated[13]. In the literature, the studies regarding the effect of naproxen on membrane dynamics also seem to reveal contradictory results. In a recent study performed by Manrique-Moreno et al. (2010), the effects of naproxen on cell membrane molecular models were investigated by DSC[10]. Their data showed a decrease in the melting temperature of dimyristoylphosphatidylcholine (DMPC) liposomes, which was attributed to a destabilization of the gel phase. These results were also supported with the data presented in another study in which the interaction of the NSAID drugs including naproxen with model membranes from synthetic lecithin was investigated[14]. Their infrared results showed that the napoxen shifts the phase transition temperature to lower values, indicating a strong fluidization of the system. In a previous calorimetric study done in 1991, it was reported that addition of increasing amounts of naproxen to dipalmitoylphosphatidylcholine (DPPC) liposomes causes a decrease in the transition temperature, associated to the gel-to-liquid crystal phase transition[15]. On the other hand, a rigidifying effect of naproxen was also reported in the literature[12].

Due to the contradictions in the literature regarding the effect of sulindac and naproxen on membrane fluidity, we aimed to investigate their concentration dependent effects on model membranes prepared from DPPC:Cholesterol as function of temperature. To accomplish this purpose, we used turbidity technique which can be applied to study drug-lipid interactions by measuring the change in the absorbance values. We have chosen DPPC as the phospholipid component of our model membrane, because phosphatidylcholines are the major lipidic components of cellular membranes, including gastric mucosa, and consequently they are the most commonly used ones in model membrane studies as the representative phospholipid of most cellular membranes[16]. Cholesterol was also included in our membrane model together with DPPC to mimic the natural membranes, because this combination can provide a closer understanding of biological membranes than the pure lipid systems. Besides, cholesterol is generally used in liposomal formulations to increase bilayer resistance to in vivo degradation[17].

EXPERIMENTAL

DPPC, cholesterol, sulindac, naproxen, ethanol, and chloroform were purchased from Sigma, USA. DPPC and cholesterol were stored at –20 ºC, sulindac and naproxen were as stored at room temperature. All other chemicals were purchased from Merck, Germany.

Sample preparation

For turbidity measurements, pure phospholipid multilamellar vesicles (MLVs) were prepared according to the procedure reported previously[18,19]. Briefly, 1.5 mg of DPPC was dissolved in 150 µL of chloroform and excess chloroform was evaporated by using a gentle stream of nitrogen. A dried lipid film was obtained by subjecting the samples to vacuum drying for 2 hours, using the HETO-spin vac system (HETO, Allerod, Denmark). The lipid films were then hydrated by adding 1.5 mL of 0.1 M phosphate-buffered saline (PBS) buffer (pH 7.4). MLVs were formed by vortexing the mixture at 60 ºC, which is above the phase transition temperature (T_m) of DPPC (which is around 41 ºC) for 20 minutes. The temperature was maintained at this specific value by immersing the tubes in a water bath at 60 °C for 2 minutes, followed by vortexing for 2 minutes. In order to prepare binary mixtures of DPPC and cholesterol, the required amount of cholesterol (DPPC:Cholesterol

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mol ratio of 1:1) from a stock solution of 5 mg/mL in chloroform was added and excess chloroform was evaporated by using a gentle stream of nitrogen. Then, the same procedure for the preparation of pure DPPC liposomes was followed. To prepare drug containing DPPC-Cholesterol liposomes, the required amount of sulindac (or naproxen) (1, 6, 9, 12, and 18 mol%) from a stock solution of 5 mg/mL in ethanol was first added to a tube, excess ethanol was evaporated by using a gentle stream of nitrogen, then 1.5 mg of DPPC was added and dissolved in 150 µL chloroform. MLVs were then prepared as described above.

Turbidity measurements

Turbidity studies were carried out by using a Varian-Cary 300 UV/Visible spectrophotometer (Varian Inc., Melbourne, Australia) as reported previously[20]. Plastic clear cuvettes (LP Italiana, Milan, Italy) of 1 cm in path length were used. To automatically account for background absorbance of the buffer, reference cuvettes were filled with PBS buffer. In order to minimize any light-scattering effect, turbidity measurements were performed at 440 nm[20]. Samples were scanned between 25 ºC and 65 ºC with 2 ºC intervals. The samples were incubated for 5 min before recording the absorbance values.

RESULTS AND DISCUSSION

A study on cholesterol containing DPPC liposomes were performed as a function of temperature to assess the fluidity changes of the membranes in the presence of different concentrations of NSAIDs, namely sulindac and naproxen, by using turbidity technique. Although this technique is not as sensitive as other spectroscopic techniques, the lower cost of the equipment, maintenance, availability, and ease of use should be noted as the advantages of the technique. In addition, the required concentrations of the samples are very low, compared to other techniques[18].

Alterations within cellular membranes often go along with changes in membrane fluidity, which is a physical parameter susceptible to perturbations by membrane active drugs. Fluidity refers to the rate of motion but not to the ordering of the molecular system[21]. Changes in this specific parameter can be measured by tracing the change in the absorbance values in turbidity technique, where an increase in absorbance is equivalent to a decrease in membrane fluidity and visa versa. The phase transition of phospholipids from the gel to the liquid crystalline phase causes a decrease in absorbance values. This is mainly due to the changes that occur in the refractive index of the lipids, as a consequence of changes in the lipid density during melting[22]. Figure 1 shows the temperature dependent variations in the absorbance values at 440 nm for pure and 50 mol% cholesterol containing DPPC liposomes (this amount mimics cholesterol content of biological membranes[23]).

As it is demonstrated in Figure 1, pure DPPC liposomes show a number of distinct thermodynamic phase transitions. With increasing temperature, the main phase transition takes the lipid bilayer from the gel to the liquid-crystalline phase. The gel phase, below the T_m, is characterized by slow translational and rotational

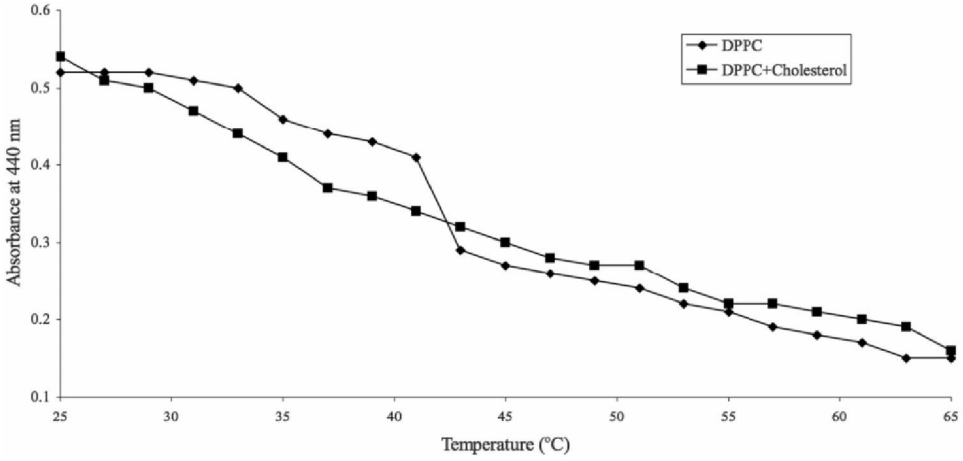

Figure 1 : Temperature dependence of the absorbance at 440 nm for pure DPPC and 50 mol% cholesterol containing DPPC liposomes

diffusion of lipid molecules. Above T_m, the lipid molecules exhibit increased diffusional mobility[19]. Figure 1 shows that, with the addition of 50 mol% cholesterol, the main phase transition completely disappears. It was reported in a previous study that the addition of cholesterol induces a new phase, called as the liquid-ordered phase, which is characterized by fluid-like membrane properties and high molecular order of the lipid chains[24]. It was also reported in the same study that, at high cholesterol concentrations (exceeding 25 mol%), the main phase transition completely disappears and the bilayer is in the liquid-ordered phase at all temperatures, which is in agreement with our current findings. Our turbidity results also reveal that the incorporation of cholesterol into pure DPPC liposomes decreases the absorbance values in the gel phase which implies its fluidizing effect in the system, and increases the absorbance values in the liquid crystalline phase, which implies its rigidifying effect (decrease in fluidity) in the system. These findings are also in close agreement with the previous studies[17]. There is a general consensus in the literature that the lipid–cholesterol interaction is dominated by the hydrophobic interaction between cholesterol and the lipid acyl chains of the phospholipids[25].

Figure 2 displays the temperature-induced changes in the absorbance values at 440 nm for DPPC-Cholesterol liposomes containing 1, 6, 9, 12, and 18 mol% sulindac.

As can be clearly seen from the Figure, the incorporation of 1 mol% sulindac does not have any significant effect on the absorbance values in the studied temperature range (25-65 ºC). Addition of 6, 9, 18 mol % sulindac increases the absorbance values suggesting that this drug has rigidifying effect in our model membrane system regardless of the temperature. Our results are in close agreement with a previous study in which it was suggested that sulindac alters the physical state of the membranes by strongly decreasing their fluidity[11]. The fluidity is expected to influence bilayer permeability property, which is required for optimal activity of membrane associated structures which in turn influence cellular processes and disease states. Sulindac may be beneficial in therapy of and prevention from cancers because of its membrane stabilizing effect (decreasing fluidity), since metastatic tumor cells have higher plasma membrane fluidity than non-metastatic cells, thus decreased membrane fluidity could restore contact inhibition between the cells by increasing the rigidity of the cells. Furthermore, cell division may be slower as a result of reduced membrane fluidity[26]. In close aggreement with our experimental findings and discussion, it was suggested in a very recent study that, sulindac might be exerting its antineoplastic role in colorectal cancer by decreasing the fluidity of the membrane toward normalizing the physical conditions in membrane[27]. The sulindac induced decrease in membrane fluidity might be explained like the following: Sulindac exists in anionic forms at pH 7.4, and it would interact with the zwitterionic groups of DPPC. As NSAIDs are likely to be present at the surface of the bilayer as it was previously suggested[28], the possible charge head group induced by NSAID surface binding and the consequent elimination of the electrostatic repulsion between polar head groups of the phospholipids may allow a better packing of the

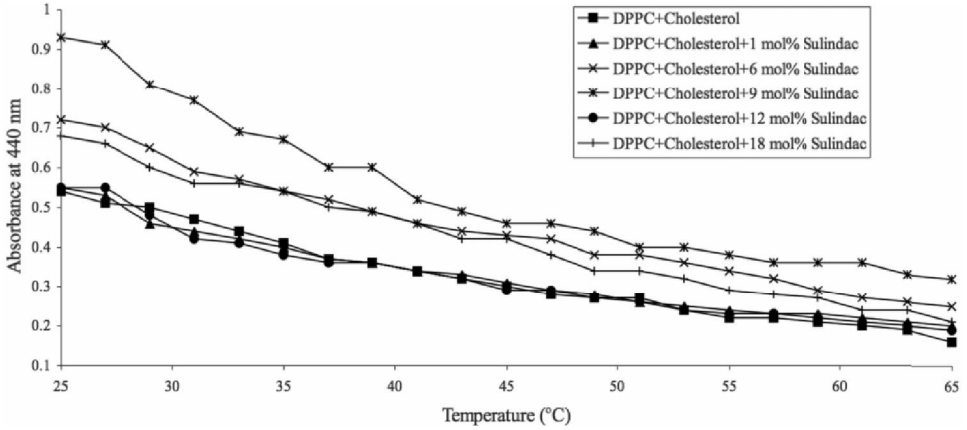

Figure 2 : Temperature dependence of the absorbance at 440 nm for 50 mol% cholesterol containing DPPC liposomes in the presence of different sulindac concentrations (1, 6, 9, 12, and 18 mol%)

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hydrocarbon chains[29]. Sulindac could reach the surface of the bilayer, most probably with the negative carboxyl group anchored near the phospholipid head group, and with the rest of the drug molecules buried partly in the membrane and aligned with the phospholipid aliphatic tails[28].

Figure 3 shows the temperature dependent changes in the absorbance values at 440 nm for DPPC-Cholesterol liposomes containing 1, 6, 9, 12, and 18 mol% naproxen.

As it is seen in the Figure, the absorbance values decrease at temperatures below 40 °C and slightly increase at higher temperatures with the addition of naproxen at all concentrations except 9 mol% (negligible effect). These findings suggest that naproxen has fluidizing effect at low temperatures (below 40 ºC) and a slight rigidifying effect at high temperatures (above 40 ºC). Destabilizing effect of naproxen in gel phase was reported in a previous DSC study in 2010[10]. Different than our study, they used DMPC liposomes without cholesterol, as their model membrane system. It was suggested in another previous study that naproxen is located within the bilayer, at a level closer to the polar head groups than the fatty acid tail[12]. In another study, Lichtenberger found that naproxen was able to interact with the zwitterionic phospholipid DPPC possibly due to hydrophobic and electrostatic interactions[30]. In an infrared study performed by Manrique Moreno et al. (2009), it was revealed that naproxen was located preferentially in the polar head groups of the phospholipids, close to the phosphate region[14]. The naproxen induced increase in the fluidity of the model membrane systems in the gel phase, which is suggested by us and the others mentioned above, might be explained like the following: Naproxen might induce changes in the packing of the polar head group region, by modifying the surface-bond water molecules in the bilayer. It is well known that the phosphate group is sensitive to hydration alterations in membranes. The hydration plays an important role in the stability of the bilayer. The alterations of the hydration shell have consequences for the membrane, like changes in the semi-permeable properties, rate and efficiency of the cell growth and modulate the activity of a variety of membrane-associated enzymes[14]. A rigidifying effect of naproxen at higher temperatures (above 40 ºC) is quite difficult to explain. But, one explanation could be the possibility of electrostatic bonding between the negatively charged carboxyl group of the naproxen and the positively charged quarternium ammonium of the phosphatidylcholine, as suggested previously[12].

In the present study, the concentration dependent effects of sulindac and naproxen on DPPC-Cholesterol model membrane system by means of lipid dynamics have been investigated for the first time. Our turbidity data suggested that both sulindac and naproxen alter the physicochemical properties of cellular membranes by changing the fluidity of the DPPC-Cholesterol model membrane system. The significance of these findings lies behind the fact that the changes in membrane fluidity is important since several integral membrane proteins, including ion channels, are affected in their function by alterations in bilayer properties. This kind of effect also has clinical importance due to the fact that NSAIDs

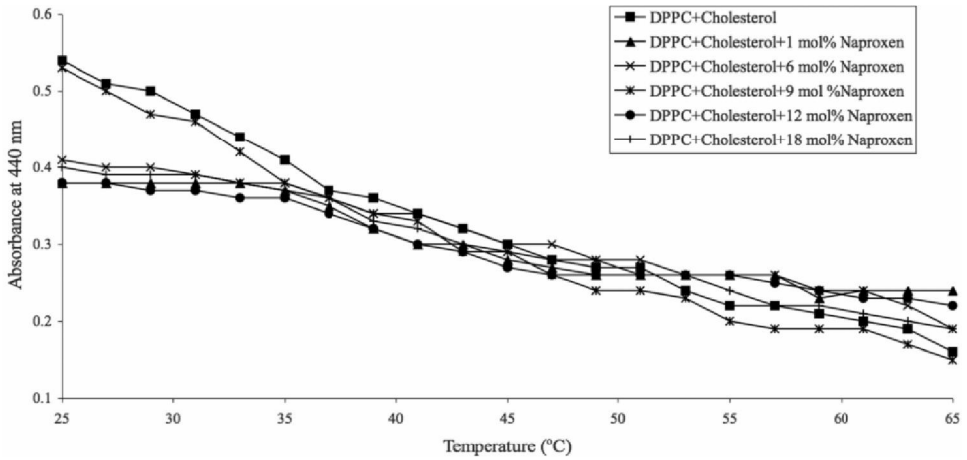

Figure 3 : Temperature dependence of the absorbance at 440 nm for 50 mol% cholesterol containing DPPC liposomes in the presence of different naproxen

induced changes in the optimal fluidity of the gastric phospholipids may reduce their ability to form a protective hydrophobic layer. The clinical significance of these observations becomes evident with regard to the development of a new formulation of NSAIDs, where the drug is pre-associated to synthetic phospholipid before administration to limit the interactions of the NSAID with the intrinsic phospholipid lining of the stomach.

CONCLUSIONS

In the present study, the effects of different concentrations of sulindac and naproxen on fluidity of the model membranes prepared from DPPC-Cholesterol was investigated by turbidity technique for the first time. Our data suggested that sulindac has rigidifying effect on the DPPC-Cholesterol model membrane system at all temperatures studied; while naproxen has fluidizing effect at low temperatures (below 40 ^{o}C) and a slight rigidifying effect at higher temperatures. Our findings suggested that both of the drugs directly alter the membrane architecture. These general membrane effects might be helpful to understand the underlying mechanism by which NSAIDs show their effect in general and specially in cancer chemotherapeutic and chemopreventive therapy. Nevertheless, further investigations are required to provide a deeper insight into the underlying mechanism that leads to changes in membrane fluidity-dynamics especially by including other techniques and other types of membrane lipids (using different phospholipid mixtures with appropriate ratios to mimic the natural membranes).

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