Spectroscopy 25 (2011) 177–185
DOI 10.3233/SPE-2011-0506
IOS Press

Effects of the non-steroidal anti-inflammatory drug celecoxib on cholesterol containing distearoyl phosphatidylcholine membranes

Asli Sade, Sreeparna Banerjee * and Feride Severcan
Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

Abstract. The effects of different concentrations of celecoxib on the acyl chain order, dynamics and the hydration status of the head group and interfacial region of model membranes containing DSPC and cholesterol were investigated in detail using Fourier transform infrared spectroscopy. Our results reveal that regardless of the presence of cholesterol, celecoxib is able to alter the physical properties of membranes. It exerts opposing effects on membrane order at high and low concentrations and decreases membrane fluidity in the presence of cholesterol. An evidence of phase separation has also been observed. The decrease in membrane fluidity supports the hypothesis that celecoxib may induce changes in the activity of membrane bound enzymes through modulating physical properties of the membrane thereby contributing to its anticancer activity. A possible change in the location of celecoxib in DSPC membranes when cholesterol is present has also been proposed. These results clarify, to a certain extent, the molecular interactions of celecoxib with membrane systems and may additionally contribute to a better understanding of COX-2 independent mechanisms of celecoxib action.

Keywords: Celecoxib, cholesterol, DSPC, FTIR, MLV

1. Introduction

Cyclooxygenases (COX), also called prostaglandin H synthases (PGHS), are enzymes localized at the nuclear or endoplasmic reticulum membrane of eukaryotic cells. These are the key enzymes of the eicosanoid cascade which can convert arachidonic acid to prostaglandins. Cyclooxygenases have two isoforms, COX-1 and COX-2, which have different expression patterns in the tissues. COX-1 is needed for a basal level of prostaglandin synthesis required for 'housekeeping' functions in the body such as gastrointestinal cytoprotection, renal functions and vascular homeostasis and is expressed constitutively in most tissues. Whereas, COX-2, is not constitutively expressed in most tissues with the exception of kidney and brain [9]. This isoform can be induced by a number of stimuli, such as bacterial lipopolysaccharide (LPS), interleukin-1 and 2, and tumor necrosis factor (TNF)-α, all of which are related to inflammation [9]. The induction of COX-2 by inflammatory mediators led investigators to label this enzyme as pathological [28].

*Corresponding author: Sreeparna Banerjee, Department of Biology, Middle East Technical University, Ankara 06531, Turkey. Tel.: +90 312 210 6468; Fax: +90 312 210 7976; E-mail: banerjee@metu.edu.tr.

Celecoxib (CLX) is a diaryl substituted pyrazole class of compound with the chemical designation (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]) benzenesulfonamide which is a new generation of non-steroidal anti-inflammatory drug (NSAID). CLX has been indicated to relieve the symptoms of osteoarthritis and rheumatoid arthritis [11]. This drug is a selective inhibitor of COX-2 which gives it the advantage of reduced gastrointestinal bleeding compared to classical NSAIDs (e.g., acetylsalicylic acid, indomethacin) [11].

According to previous epidemiological studies, long term CLX usage is related to a chemopreventive activity in colorectal [3], breast [13] and lung carcinogenesis [14]. CLX is the only NSAID that has been approved by the FDA for supplementary treatment of familial adenomatous polyposis currently. The mechanisms underlying the anti-carcinogenic nature of CLX are not completely understood and several cancer related pathways are known to be affected by CLX independently of COX-2 [12]. CLX has been shown to induce cell cycle arrest, apoptosis and to inhibit angiogenesis and metastasis by influencing either the expression or the activity of related proteins [12]. Some direct targets of celecoxib such as protein kinase B or phosphoinositide-dependent kinase 1 has been proposed. In addition, celecoxib was found to inhibit the activity of some membrane bound proteins such as Ca^{2+} ATPase, however the action mechanism is not clear [12].

Celecoxib has also been shown to have an uncharacterized role in Alzheimer's disease. In addition, some COX independent mechanisms, such as membrane dynamics and structure, have also been suggested for its activity [10]. It was proposed that celecoxib induces formation of amyloid-β peptides by ordering the cellular membranes and thus changing the activity of membrane bound proteins, especially β- and γ-secretase. Therefore, it is not clear whether CLX exerts its function through a direct interaction with membrane bound enzymes or whether its interaction with cellular membranes alters the activity of these enzymes. Amphipathic and lipophilic compounds are not only able to alter the phase transition, fluidity and order of the membrane lipids but also membrane function which is achieved by influencing the activity of integral membrane proteins [16,17,19,20]. CLX, being a lipophilic compound, is also a potential modulator of membrane lipid structure and dynamics and understanding these interactions at molecular level will provide further understanding of its mechanism of action.

Despite its importance, studies on the interaction of CLX with membranes at a molecular level are very limited. Walter et al. [32] showed that CLX is localized at the interfacial region of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) – cholesterol membranes using small angle X-ray diffraction. In other studies, using fluorescence anisotropy technique, CLX has been shown to decrease membrane fluidity in a mouse neuroblastoma cell line N2a [10] and decrease in membrane fluidity in egg phosphatidylcholine model membranes [27]. In our previous study, using Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC), we have found that CLX exerts opposing effects on membrane order in a concentration dependent manner and it decreases the fluidity of the membrane at all concentrations. We have also proposed a possible location of CLX at low concentrations in the interfacial region and at high concentrations in the deeper hydrophobic region of the membrane [22].

In the present study, we aimed to further investigate the COX-2 independent membrane modulating effects of different concentrations of CLX particularly in the presence of cholesterol. In order to mimic a biological membrane which usually contains a cholesterol to phospholipid ratio of 0.11–0.33 [7], we have chosen a DSPC:Chol ratio of 3:1 (0.25) for our investigation. Therefore, the effect of high and low concentrations of CLX on model membranes composed of a zwitterionic lipid, 1,2-Distearoyl-sn-Glycero-3-Phosphocholine (DSPC) and cholesterol, in the form of multilamellar vesicles (MLVs) has been investigated using Fourier transform infrared (FTIR) spectroscopy. Presence of cholesterol in our model membrane system allowed us to further mimic a biological membrane and to obtain detailed

information about the concentration dependent effects of CLX on membrane dynamics and structural parameters.

2. Materials and methods

2.1. Materials

DSPC and cholesterol were purchased from Avanti Polar Lipids (Alabaster, AL, USA) and stored at −20°C. CLX was purchased from Ranbaxy (Mumbai, India). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Sample preparation

MLVs were prepared according to the procedure reported previously [17,26]. Briefly, 5 mg of DSPC and 0.82 mg cholesterol (to have a DSPC:Chol molar ratio of 3:1) was dissolved in 150 µl 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 h using HETO-spin vac system (HETO, Allerod, Denmark). The lipid films were then hydrated by adding 35 µl of 0.1 M phosphate buffered saline (PBS) buffer, pH 7.4. MLVs were formed by vortexing the mixture at 70–75°C which is above the T_m of DSPC (∼55°C) for 20 min. The temperature was maintained by immersing the tubes in a water bath at 75°C for 2 min followed by vortexing for 2 min. This was repeated 5 times. To prepare CLX containing liposomes, the required amount of CLX (6 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 DSPC and cholesterol were added and dissolved in 150 µl chloroform. MLVs were then prepared as described above.

For FTIR measurements, 20 µl of sample suspensions in PBS buffer (0.1 M, pH 7.4) were placed between CaF_2 windows with a cell thickness of 12 µm and immediately placed in the sample holder of the FTIR equipment.

2.3. FTIR measurements and spectral analysis

Spectra were recorded on a Perkin–Elmer Spectrum One FTIR spectrometer (Perkin–Elmer Inc., Norwalk, CT, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. Interferograms were averaged for 50 scans at 2 cm^{-1} resolution. The samples incubated at 30 and 65°C for 10 min and were scanned at those temperatures. The temperatures were decided according to previous DSC measurements [8] for the correct monitoring of the gel and liquid crystalline phases. The temperature was controlled by a Graseby Specac (Kent, UK) digital temperature controller unit.

The spectra were analyzed using Spectrum v5.0.1 software (Perkin–Elmer Inc., Norwalk, CT, USA). In order to provide a better resolution of the infrared bands, 0.1 M PBS buffer (pH 7.4) bands were subtracted by flattening the band located around 2125 cm^{-1}. The band positions were measured according to the center of weight and bandwidth was measured at 0.80× peak height position.

2.4. Statistical analyses

Data analysis and graphing was performed using the GraphPad Prism 5 software package (La Jolla, CA, USA). Specific analysis for each experiment is indicated in the figure legends. Unless otherwise mentioned, the mean of at least five experiments was plotted together with the standard error of mean.

Statistical significance was assessed using a two-tailed p-value calculated with the Mann–Whitney non-parametric test. Significant difference was statistically considered at the level of $p \leqslant 0.05$.

3. Results and discussion

In this study, FTIR spectroscopy was used to determine the changes in membrane structure and dynamics in response to low and high concentrations of CLX by analyzing the frequency and bandwidth of different vibrational modes which represent the acyl chains, head group and interfacial region of lipid molecules [5,26].

The strong bands at 2920 and 2850 cm^{-1} of the infrared spectrum correspond to the CH_2 antisymmetric and CH_2 symmetric stretching modes of the acyl chains of the phospholipid membranes, respectively. The frequency of these bands give information about the order/disorder state of the membrane depending on the average trans/gauche isomerization in the system, and shifts to higher wavenumbers correspond to an increase in the number of gauche conformers which implies a more disordered state [5,23]. Figure 1 displays the average frequency changes in the CH_2 antisymmetric stretching mode of DSPC:Chol MLVs (mol ratio of 3:1), in the presence and absence of 6 and 18 mol% CLX at 30 and 65°C. These CLX concentrations were chosen according to our previous findings which indicated that 6 and 18 mol% CLX show opposing effects on DSPC model membranes [22]. As seen from the figure, in the gel phase, the frequency significantly shifts to lower values with the incorporation of 6 mol% CLX, indicating an increase in the number of trans conformers thus increasing the order of the system. 18 mol% CLX, on the other hand, causes a significant frequency shift to higher values, which indicates a decrease in the order of the membranes. However in the liquid crystalline phase, an increase in the frequency is seen for 18 mol% CLX, whereas no significant difference was observed for 6 mol% CLX. This indicates that in the liquid crystalline phase CLX is effective only at high concentrations. Therefore, when cholesterol is present in the DSPC membrane system, the opposing effect of high and low concentrations of CLX on

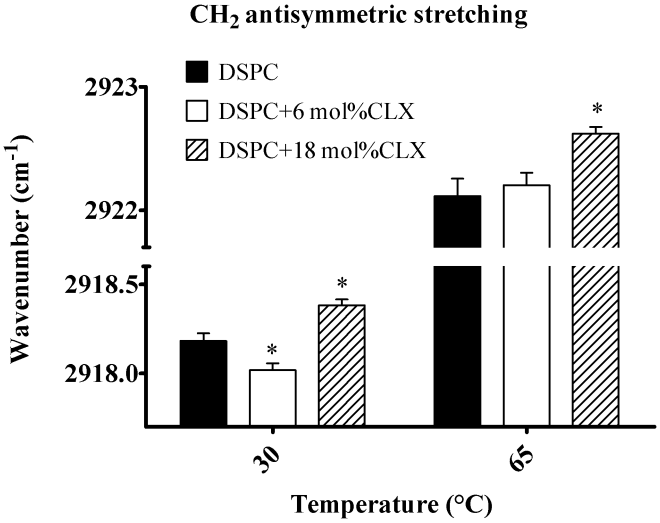

Fig. 1. Average frequency changes in the CH_2 antisymmetric stretching mode of DSPC:Chol MLVs (mol ratio of 3:1) in the presence and absence of 6 and 18 mol% CLX at 30 and 65°C. Each point represents the mean ± SEM ($n = 5$). $^{*}p < 0.05$ compared to controls (0 mol% CLX).

membrane order can only be observed in the gel phase. Observing no effect for 6 mol% CLX in the liquid crystalline phase may have resulted from the effect of cholesterol on the membrane system. It has been widely accepted and also observed in our studies (data not shown) that cholesterol exerts opposite effects on the acyl chain conformation below and over the T_m of phospholipids; disordering the membrane in the gel phase and ordering it in the liquid crystalline phase [1,24,31,33]. When the ternary system of DSPC:Chol:CLX is considered, the ordering effect of cholesterol in the liquid crystalline phase most probably have surpassed the similar effect of 6 mol% CLX resulting in no significant difference.

Information about the dynamics of membrane systems can be obtained by analyzing the variations in the bandwidth of the CH_2 stretching modes, since bandwidth reflects the changes in the mobility of the acyl chains. An increase in the bandwidth is an indication of an increase in the dynamics and therefore the fluidity of the membrane system [4,17,18,25,26]. In the interpretation of the results in FTIR spectra, extra care was taken in distinguishing between structural parameters (frequency) describing molecular order and dynamical parameters (bandwidth) describing molecular mobility, as suggested previously by us and others [26,29]. The variations in the bandwidth of the CH_2 antisymmetric stretching mode of DSPC:Chol MLVs for 6 and 18 mol% celecoxib at 30 and 65°C, are given in Fig. 2. A decrease in the bandwidth with increasing concentrations of CLX (statistically significant at 18 mol%) in both phases can be observed. This indicates that CLX decreases the fluidity of DSPC:Chol membranes which is also consistent with our previous results of DSPC membranes without cholesterol [22].

The changes in the frequency and bandwidth of the CH_2 stretching mode are not concerted for high celecoxib concentrations since it induces a decrease in both membrane order and dynamics. This kind of disconcerted behavior has been previously reported for interactions of model membranes with melatonin [26], progesterone [17], vitamin D_2 [15], vitamin E [30] and cholesterol [29]. This controversial effect of high celecoxib concentrations on membrane order and dynamics may reflect the presence of more than one phase in the bilayer [30]. This is in agreement with our previous drug delivery study in which DSPC liposome formulations containing CLX and different amounts of cholesterol (2:1, 5:1 and 10:1 phospholipid:cholesterol molar ratio) were found to produce new domains in the membranes [8].

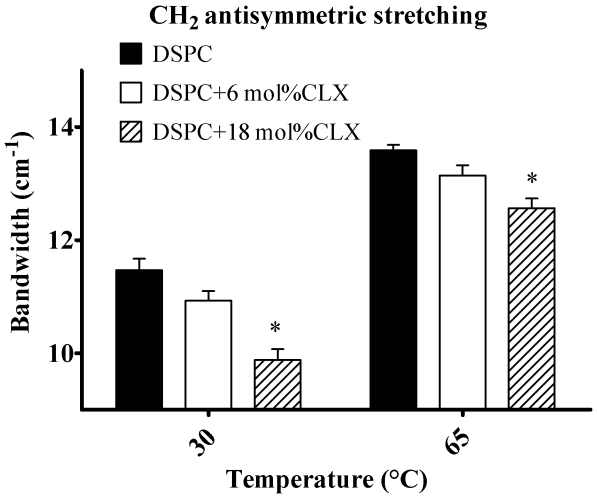

Fig. 2. Average bandwidth changes in the CH_2 antisymmetric stretching mode of DSPC:Chol MLVs (mol ratio of 3:1) in the presence and absence of 6 and 18 mol% CLX at 30 and 65°C. Each point represents the mean ± SEM ($n = 5$). $^*p < 0.05$ compared to controls (0 mol% CLX).

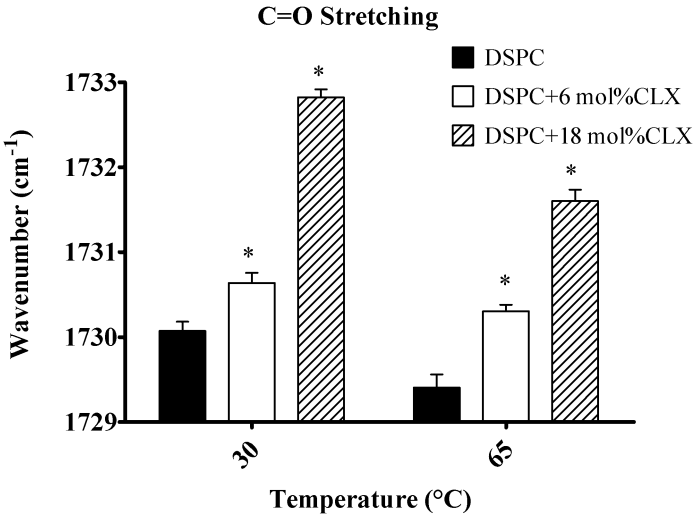

Fig. 3. Average frequency changes in the C=O stretching mode of DSPC:Chol MLVs (mol ratio of 3:1) in the presence and absence of 6 and 18 mol% CLX at 30 and 65°C. Each point represents the mean ± SEM ($n = 5$). $^{*}p < 0.05$ compared to controls (0 mol% CLX).

We therefore confirmed this finding in a more physiologically relevant model for both gel and liquid crystalline phases.

To get information about the effect of the drug on the region near the polar head groups of the DSPC:Chol membranes, carbonyl absorption (C=O stretching) band at 1735 cm^{-1} was monitored. This band arises from the stretching vibrations of ester carbonyl groups of phospholipids and is conformationally sensitive to the level of hydration at the membrane interface [17,26]. Therefore any effect seen in the spectra of this region (C=O stretching) can be attributed to an interaction of celecoxib or cholesterol with the polar/apolar interfacial region of the membrane. Figure 3 illustrates the average changes in the frequency of the C=O stretching mode of DSPC:Chol MLVs, in the presence and absence of 6 and 18 mol% celecoxib, at 30 and 65°C. CLX induces an increase in the frequency of this band in both phases indicating a decrease in hydration around the carbonyl groups of phospholipids. This is explained by the presence of more free carbonyl groups in the system when there is CLX, thus no evidence of hydrogen bonding between CLX and the C=O group is observed. This result is controversial to our previous finding that states CLX at low concentrations makes hydrogen bonds with the carbonyl groups in DSPC membranes (without cholesterol). In addition, cholesterol has also been found to induce hydration around this region [8] and one would expect a synergistic effect when both CLX and cholesterol are present in the system. However, it was also stated by our group that cholesterol at high concentrations (DSPC:Chol mol ratio of 2:1) locates itself close to glycerol backbone and thereby confines CLX (18 mol%) to the deeper interior of the membrane, forming a new cooperative domain in the membrane [8]. Moreover, cholesterol has been known to have a higher probability to bond with C=O rather than the PO_2^- groups of phospholipids [1,6,21,34]. Combining this knowledge with the present findings led us to conclude that CLX at low concentrations (6 mol%) may also be subjected to a similar kind of entrapment in the deeper core of the membrane which may explain the dehydration around the carbonyl groups.

Information about the hydration state of the polar head groups of the phospholipids can be monitored by the analysis of the frequency of the PO_2^- antisymmetric double stretching band, located at 1220–1240 cm^{-1} [17,18,26]. The variations in the frequency of the PO_2^- antisymmetric double stretching

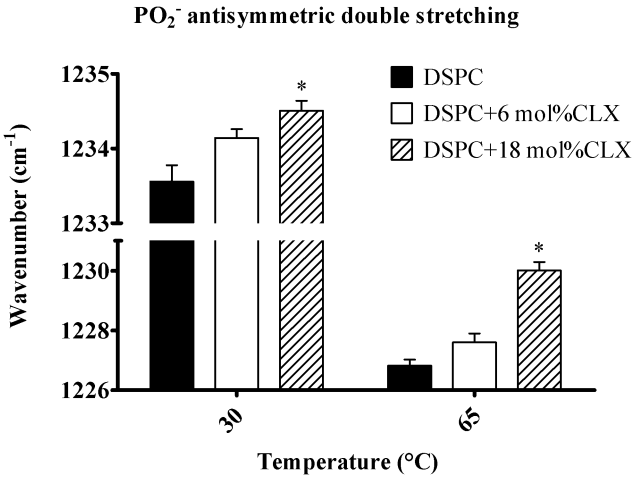

Fig. 4. Average frequency changes in the PO_2^- antisymmetric double stretching mode of DSPC:Chol MLVs (mol ratio of 3:1) in the presence and absence of 6 and 18 mol% CLX at 30 and 65°C. Each point represents the mean ± SEM ($n = 5$). $^*p < 0.05$ compared to controls (0 mol% CLX).

mode of DSPC:Chol MLVs for 6 and 18 mol% celecoxib at 30 and 65°C, are given in Fig. 4. Incorporation of CLX induces a shift of the frequency to higher values which indicates an increase in the dehydration around the head groups and presence of free PO_2^- groups. This result is in agreement with our previous findings in which there is no evidence of hydrogen bonding between the head groups of phospholipids and CLX. Presence of free PO_2^- groups may also be explained by the displacement of cholesterol with hydrogen bonded water molecules in the head group region which would further support the presence of CLX in the inner core of the membrane.

4. Conclusion

In the present study the effects of different concentrations of celecoxib on the acyl chain order, dynamics and the hydration status of the head group and interfacial region of model membranes containing DSPC and cholesterol are investigated in detail. In the presence of physiologically relevant cholesterol concentration, the opposing character of high and low concentrations CLX on membrane order is still valid. Similar to non-cholesterol DSPC membranes, CLX has been found to decrease membrane fluidity and induce phase separation in the presence of cholesterol. Different from the non-cholesterol system, the membrane ordering effect of low CLX concentrations was diminished by the presence of cholesterol in the liquid crystalline phase. However, regardless of the presence of cholesterol, CLX is still able to alter the physical properties of membranes. This information is important because any variation in lipid structure is known to influence permeability and result in functional changes in membrane proteins [16, 19,20]. In addition, membrane lipid order and fluidity have been reported to be important parameters for the correct functioning of ion channels [2]. The decrease in membrane fluidity in a more biologically relevant system containing cholesterol supports the hypothesis that CLX may induce changes in the activity of membrane bound enzymes through modulating physical properties of the membrane thereby contributing to its anticancer activity [22].

In this study, a possible change in the location of CLX in DSPC membranes when cholesterol is present has also been proposed. Although CLX, at low concentrations, tends to hydrogen bond with

the carbonyl groups and locates itself close to this moiety, cholesterol, being a larger molecule, may be capable of locating CLX deeper into the hydrophobic core of the membrane.

As a conclusion, these results further delineate the molecular interactions of CLX with membrane systems and may contribute to a better understanding of COX-2 independent mechanisms of celecoxib action.

Acknowledgements

The study was funded by the Scientific Research Council of Middle East Technical University Project Nos 2007-(R)-08-11-02 and BAP-07.02.2009.04.

References

[1] Z. Arsov and L. Quaroni, Direct interaction between cholesterol and phosphatidylcholines in hydrated membranes revealed by ATR–FTIR spectroscopy, *Chemistry and Physics of Lipids* **150** (2007), 35–48.
[2] M.S. Awayda, W.J. Shao, F.L. Guo, M. Zeidel and W.G. Hill, ENaC-Membrane interactions: Regulation of channel activity by membrane order, *Journal of General Physiology* **123** (2004), 709–727.
[3] M.M. Bertagnolli, C.J. Eagle, A.G. Zauber, M. Redston, S.D. Solomon, K.M. Kim, J. Tang, R.B. Rosenstein, J. Wittes, D. Corle, T.M. Hess, G.M. Woloj, F. Boisserie, W.F. Anderson, J.L. Viner, D. Bagheri, J. Burn, D.C. Chung, T. Dewar, T.R. Foley, N. Hoffman, F. Macrae, R.E. Pruitt, J.R. Saltzman, B. Salzberg, T. Sylwestrowicz, G.B. Gordon, E.T. Hawk and APC Study Investigators, Celecoxib for the prevention of sporadic colorectal adenomas, *New England Journal of Medicine* **355** (2006), 873–884.
[4] H.L. Casal, D.G. Cameron, I.C.P. Smith and H.H. Mantsch, Acholeplasma-laidlawii membranes – Fourier-transform infrared study of the influence of protein on lipid organization and dynamics, *Biochemistry* **19** (1980), 444–451.
[5] H.L. Casal and H.H. Mantsch, Polymorphic phase-behavior of phospholipid-membranes studied by infrared-spectroscopy, *Biochimica et Biophysica Acta* **779** (1984), 381–401.
[6] C.F. Chen and C.P. Tripp, An infrared spectroscopic based method to measure membrane permeance in liposomes, *Biochimica et Biophysica Acta – Biomembranes* **1778** (2008), 2266–2272.
[7] R.A. Demel and B. Dekruyff, Function of sterols in membranes, *Biochimica et Biophysica Acta* **457** (1976), 109–132.
[8] A. Deniz, A. Sade, F. Severcan, D. Keskin, A. Tezcaner and S. Banerjee, Celecoxib loaded liposomes: effect of cholesterol on encapsulation and *in vitro* release characteristics, *Bioscience Reports* **30** (2010), 365–373.
[9] C.D. Funk, Prostaglandins and leukotrienes: Advances in eicosanoid biology, *Science* **294** (2001), 1871–1875.
[10] M. Gamerdinger, A.B. Clement and C. Behl, Cholesterol-like effects of selective cyclooxygenase inhibitors and fibrates on cellular membranes and amyloid-beta production, *Molecular Pharmacology* **72** (2007), 141–151.
[11] M.M. Goldenberg, Celecoxib, a selective cyclooxygenase-2 inhibitor for the treatment of rheumatoid arthritis and osteoarthritis, *Clinical Therapeutics* **21** (1999), 1497–1513.
[12] S. Grosch, T.J. Maier, S. Schiffmann and G. Geisslinger, Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors, *Journal of the National Cancer Institute* **98** (2006), 736–747.
[13] R.E. Harris, J. Beebe-Donk and G.A. Alshafie, Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors, *BMC Cancer* **6** (2006).
[14] R.E. Harris, J. Beebe-Donk and G.A. Alshafie, Reduced risk of human lung cancer by selective cyclooxygenase 2 (Cox-2) blockade: Results of a case control study, *International Journal of Biological Sciences* **3** (2007), 328–334.
[15] N. Kazanci, N. Toyran, P.I. Haris and F. Severcan, Vitamin D-2 at high and low concentrations exert opposing effects on molecular order and dynamics of dipalmitoyl phosphatidylcholine membranes, *Spectroscopy – An International Journal* **15** (2001), 47–55.
[16] R.A. Knazek, S.C. Liu, J.R. Dave, R.J. Christy and J.A. Keller, Indomethacin causes a simultaneous decrease of both prolactin binding and fluidity of mouse-liver membranes, *Prostaglandins and Medicine* **6** (1981), 403–411.
[17] F. Korkmaz and F. Severcan, Effect of progesterone on DPPC membrane: Evidence for lateral phase separation and inverse action in lipid dynamics, *Archives of Biochemistry and Biophysics* **440** (2005), 141–147.
[18] F. Lopezgarcia, V. Micol, J. Villalain and J.C. Gomezfernandez, Infrared spectroscopic study of the interaction of diacylglycerol with phosphatidylserine in the presence of calcium, *Biochimica et Biophysica Acta* **1169** (1993), 264–272.
[19] M. Lucio, H. Ferreira, J. Lima, C. Matos, B. de Castro and S. Reis, Influence of some anti-inflammatory drugs in membrane fluidity studied by fluorescence anisotropy measurements, *Physical Chemistry Chemical Physics* **6** (2004), 1493–1498.

[20] F.R. Maxfield and I. Tabas, Role of cholesterol and lipid organization in disease, *Nature* **438** (2005), 612–621.
[21] T.J. McIntosh, Effect of cholesterol on structure of phosphatidylcholine bilayers, *Biochimica et Biophysica Acta* **513** (1978), 43–58.
[22] A. Sade, S. Banerjee and F. Severcan, Concentration-dependent differing actions of the nonsteroidal anti-inflammatory drug, celecoxib, in distearoyl phosphatidylcholine multilamellar vesicles, *Journal of Liposome Research* **20** (2010), 168–177.
[23] F. Severcan, H.O. Durmus, F. Eker, B.G. Akinoglu and P.I. Haris, Vitamin D-2 modulates melittin-membrane interactions, *Talanta* **53** (2000), 205–211.
[24] F. Severcan, N. Kazanci, U. Baykal and S. Suzer, IR and turbidity studies of vitamin-E–cholesterol–phospholipid membrane interactions, *Bioscience Reports* **15** (1995), 221–229.
[25] F. Severcan, N. Kazanci and F. Zorlu, Tamoxifen increases membrane fluidity at high concentrations, *Bioscience Reports* **20** (2000), 177–184.
[26] F. Severcan, I. Sahin and N. Kazanci, Melatonin strongly interacts with zwitterionic model membranes – evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry, *Biochimica et Biophysica Acta – Biomembranes* **1668** (2005), 215–222.
[27] W. Tomisato, K. Tanaka, T. Katsu, H. Kakuta, K. Sasaki, S. Tsutsumi, T. Hoshino, M. Aburaya, D.W. Li, T. Tsuchiya, K. Suzuki, K. Yokomizo and T. Mizushima, Membrane permeabilization by non-steroidal anti-inflammatory drugs, *Biochemical and Biophysical Research Communications* **323** (2004), 1032–1039.
[28] J.R. Vane, Y.S. Bakhle and R.M. Botting, Cyclooxygenases 1 and 2, *Annual Review of Pharmacology and Toxicology* **38** (1998), 97–120.
[29] G. Vanginkel, H. Vanlangen and Y.K. Levine, The membrane fluidity concept revisited by polarized fluorescence spectroscopy on different model membranes containing unsaturated lipids and sterols, *Biochimie* **71** (1989), 23–32.
[30] J. Villalain, F.J. Aranda and J.C. Gomezfernandez, Calorimetric and infrared spectroscopic studies of the interaction of alpha-tocopherol and alpha-tocopheryl acetate with phospholipid-vesicles, *European Journal of Biochemistry* **158** (1986), 141–147.
[31] M.R. Vist and J.H. Davis, Phase-equilibria of cholesterol dipalmitoylphosphatidylcholine mixtures – H-2 nuclear magnetic-resonance and differential scanning calorimetry, *Biochemistry* **29** (1990), 451–464.
[32] M.F. Walter, R.F. Jacob, C.A. Day, R. Dahlborg, Y.J. Weng and R.P. Mason, Sulfone COX-2 inhibitors increase susceptibility of human LDL and plasma to oxidative modification: comparison to sulfonamide COX-2 inhibitors and NSAIDs, *Atherosclerosis* **177** (2004), 235–243.
[33] P.L. Yeagle, Cholesterol and the cell-membrane, *Biochimica et Biophysica Acta* **822** (1985), 267–287.
[34] P.L. Yeagle, W.C. Hutton, C.H. Huang and R.B. Martin, Headgroup conformation and lipid–cholesterol association in phosphatidylcholine vesicles – P-31(H-1) nuclear overhauser effect study, *Proceedings of the National Academy of Sciences of the United States of America* **72** (1975), 3477–3481.