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Membrane Interactivity of Non-steroidal Anti-inflammatory Drugs: A Literature Review

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Authors' contributions

This work was carried out in collaboration between both authors. Author HT designed and conducted the present study. Author HT did literature search, information analysis and manuscript preparation in collaboration with author MM. Both authors reviewed and approved the final manuscript.

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ABSTRACT

Background: Although the mode of action of non-steroidal anti-inflammatory drugs (NSAIDs) has been exclusively referred to as inhibition of cyclooxygenase, their broad pharmacological and toxicological spectra are not necessarily interpreted by the direct interaction with such enzyme proteins.
Aims: Since NSAIDs have the common amphiphilic structure, they have the possibility of acting on membrane-constituting lipids. In order to gain insights into the additional mechanism of NSAIDs, we reviewed their membrane interactivity to modify the physicochemical properties of membranes.
Methodology: We retrieved scientific articles from PubMed/MEDLINE, Google Scholar and ACS Publications by searching databases from 1990 to 2019. Research papers published in English in the internationally recognized journals and on-line journals were cited with preference to more recent publications. Collected articles were reviewed by title, abstract and text for relevance.
Results: Results of the literature search indicated that NSAIDs structure-specifically cause the *in vitro* and *in vivo* interactions with artificial and biological membranes to change membrane fluidity,

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lipid phase transition and permeability. The features and potencies of their membrane interactivity vary depending on drug concentration, medium pH and membrane lipid composition. In addition to membrane proteins, NSAIDs act on membrane lipids to exhibit the anti-inflammatory and anti-tumor activity by interacting with lipid bilayer membranes at relatively low concentrations to decrease membrane fluidity and thereby affect the enzymatic activity of membrane-associated proteins and to exhibit the gastrointestinal and cardiovascular toxicity by interacting with membranous phospholipids at relatively high concentrations to increase membrane fluidity and thereby impair the membrane-relevant biofunctions. Other diverse effects of NSAIDs may also be related to their membrane interactions.

Conclusion: NSAIDs share the membrane interactivity common to them as one of possible pharmacological and toxicological mechanisms.

Keywords: Non-steroidal anti-inflammatory drug; mechanism; membrane lipid; membrane interactivity; fluidity; lipid phase transition; permeability; cyclooxygenase.

ABBREVIATIONS

2-AS, 2-(9-anthroyloxy)stearic acid; 6-AS, 6-(9-anthroyloxy)stearic acid; 9-AS, 9-(9-anthroyloxy)stearic acid; 12-AS, 12-(9-anthroyloxy)stearic acid; 16-AP, 16-(9-anthroyloxy) palmitic acid; CL, cardiolipin; COX, cyclooxygenase; DMH, 1,2-dimethylhydrazine; DMPC, 1,2-dimyristoylphosphatidylcholine; DMPE, 1,2-dipalmitoylphosphatidylethanolamine; DOPC, 1,2-dioleoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, 1,2-dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; DSPC, 1,2-distearoylphosphatidylcholine; ESR, electron spin resonance; EYPA, egg yolk phosphatidic acid; EYPC, egg yolk phosphatidylcholine; FA, fluorescence anisotropy; FP, fluorescence polarization; FTIR, Fourier-transform infrared; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 5-LOX, 5-lipoxygenase; MIC, minimum inhibitory concentration; MOPS, 3-(N-morpholino)propanesulfonic acid; NSAID, non-steroidal anti-inflammatory drug; PBS, phosphate-buffered saline; PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPI, 1-palmitoyl-2-oleoylphosphatidylinositol; POPS, 1-palmitoyl-2-oleoylphosphatidylserine; ROS, reactive oxygen species; SM, sphingomyelin; TES, 2-[tris(hydroxymethyl)methylamine]-1-ethanesulfonic acid; Tm, phase transition temperature; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; Tris/HCl, tris(hydroxymethyl)amino-methane/HCl.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of over-the-counter and prescribed medicines most frequently used all over the world because they have anti-inflammatory, analgesic and antipyretic effects that are effectively used in the treatment of arthritis, headache, pyrexia, gout and post-operative dental pain. Evidence is growing for their anti-tumor effects on colonic, gastrointestinal, esophageal, pulmonary, prostate and skin cancers. Besides these beneficial activities, NSAIDs show diverse pharmacological properties to inhibit the growth of bacteria and fungi [1], induce apoptosis in gastric mucosal cells and colon cancer cells [2] and disturb platelet function and hemostasis [3]. On the other hand, the use of NSAIDs has the potential risk to cause gastrointestinal complications (including inflammatory injury, ulceration and bleeding) and cardiovascular events (including myocardial infarction, myocardial ischemia and abnormal bleeding tendency). The mode of action of NSAIDs has been exclusively referred to as inhibition of cyclooxygenase (COX) that catalytically biosynthesizes prostanoids from arachidonic acid. The produced prostaglandins play a critical role not only in pathological processes such as inflammation, pain, fever and tumor growth but also in physiological functions such as gastrointestinal mucosa protection and renal homeostasis regulation. Despite the frequent use of NSAIDs, the detailed mechanism(s) underlying their broad pharmacological and toxicological spectra are not necessarily clear.

After administered orally and topically, NSAIDs are required to pass across biomembranes to be absorbed and thereafter reach the site of action. The passage across membrane lipid bilayers is a

determinant of the pharmacokinetics and pharmacodynamics of NSAIDs. Since COX is an integral monotopic membrane protein that inserts into the single face of lipid bilayers [4] and its fatty acid substrate readily penetrates into membranes, the COX-mediated reactions take place in membrane lipid environments [5]. Although NSAIDs include a variety of chemicals, they have the common amphiphilic structure that would enable them to interact with membranes as well as other amphiphilic drugs [6]. In addition to enzyme proteins, therefore, NSAIDs are expected to act on membrane-constituting lipids to modify the physicochemical properties of membranes with the resultant inhibition of the enzymatic activity of membrane-associated proteins or affect the physiological property of membranous lipids with the resultant impairment of their relevant biofunctions. Drug-induced changes in membrane fluidity, lipid phase transition and permeability can influence the location and activity of membrane-bound or membrane-embedded proteins such as receptors, ion channels and enzymes [7]. In particular, membrane fluidity closely relates to the activity of membrane-associated enzymes [8-10].

The purpose of the present study is to review the membrane interactivity of NSAIDs by searching scientific articles from a mechanistic point of view in order to gain insights into the additional mode of action of NSAIDs. The focus of our review is to address whether NSAIDs exhibit the *in vitro* and *in vivo* membrane interactivity and how NSAIDs change the physicochemical membrane properties through the interactions with lipid bilayer membranes and membranous lipids. Excellent review papers were recently published for the *in vitro* assessment of the membrane interactivity of NSAIDs by Pereira-Leite et al. [11] and for the interactions of NSAIDs with membranous lipids relating to their gastrointestinal injuries by Lichtenberger et al. [12].

2. METHODS

The present review is based on published articles and information that were retrieved from PubMed/MEDLINE, Google Scholar and ACS Publications by searching databases from 1990 to 2019. The publications earlier than 1990 were exceptionally cited if they are essential for advancing the discussion. Research papers published in English in the internationally recognized journals and on-line journals were preferred, but review articles were also included when they are helpful for understanding the conventional mode of action of NSAIDs. However, non-English language citations were excluded. The literature searches were carried out by using the following terms or combinations thereof: “non-steroidal anti-inflammatory drug”, “NSAID”, “mechanism”, “membrane interactivity”, “membrane fluidity”, “membrane microviscosity”, “membrane lipid phase transition”, “membrane permeability”, “cyclooxygenase”, “COX”, “COX-2 selectivity”, “anti-inflammatory”, “anti-tumor”, “gastrointestinal toxicity”, “cardiovascular toxicity”, “antimicrobial”, “apoptosis”, “stereoisomer”, and “stereostructure-specific”. Collected articles were reviewed by title, abstract and text for relevance with preference to more recent publications. Their bibliographies were also searched for additional references.

3. RESULTS AND DISCUSSION

Results of the literature search indicated that NSAIDs cause the *in vitro* and *in vivo* interactions with membranes to change membrane fluidity, lipid phase transition and permeability. Table 1 summarizes the membrane interactions reported for NSAIDs, including investigated membranes, drug concentrations, experimental conditions and induced membrane effects.

The membrane interactions of drugs have been investigated by a variety of methodologies. Although their details are outside the scope of this review, a brief description is added to facilitate understanding of the results of actual experiments. Representative methods include fluorescence polarization (FP) or fluorescence anisotropy (FA), Fourier-transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), electron spin resonance (ESR) spectroscopy and their complementary combination [13]. Drug-induced changes in membrane fluidity (reciprocal of microviscosity) are determined by measuring FP or FA, FTIR spectroscopy and ESR spectroscopy; those in membrane lipid phase transition by measuring FP or FA and DSC and those in membrane permeability by analyzing fluorophores released from calcein-loaded membranes and fluorescence intensity decrease of Tb/dipicolinic acid co-encapsulated liposomes. FP and FA experiments have been most widely used because they can easily simulate *in vivo* conditions by using artificial membranes. The polarization of fluorescence emitted by a fluorophore incorporated into lipid bilayers

Table 1. Membrane interactions of non-steroidal anti-inflammatory drugs

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
Nimesulide Mefenamic acid Flufenamic acid Celecoxib	Liposomal membranes prepared with EYPC or EYPC plus cholesterol (10 : 7.5 in molar ratio)	10-100 μM	In HEPES buffer of pH 7.4	Decreased membrane fluidity with the potency being nimesulide > mefenamic acid > flufenamic acid > celecoxib Celecoxib, mefenamic acid and flufenamic acid increased membrane fluidity at 100 μM in the presence of 43 mol% cholesterol	[14]
Nimesulide Indomethacin Tolmetin	Large unilamellar vesicles prepared with EYPC (0.5 mM)	Nimesulide: ~180 μM Indomethacin: ~600 μM Tolmetin: ~600 μM	In HEPES buffer of pH 7.4	Nimesulide decreased membrane fluidity at ≥4 μM most potently, followed by indomethacin and tolmetin Acted on the center of lipid bilayers most intensively	[15]
Diclofenac	Large unilamellar vesicles prepared with EYPC (0.5 mM)	50-400 μM	In HEPES buffer of pH 7.4 at 25°C	Decreased membrane fluidity by acting preferentially on the membrane surface	[16]
Ibuprofen	Small unilamellar vesicles prepared with DOPC (0.1 mM)	Not specified	In phosphate buffer of pH 6.7 at 30°C	Decreased membrane fluidity	[17]
Indomethacin Ibuprofen	Unilamellar vesicles prepared with POPC, POPE, POPS, SM and cholesterol (36 : 22 : 3.5 : 3.5 : 35 in molar ratio)	Indomethacin: 100 μM Ibuprofen: 200 μM	In phosphate buffer of pH 7.4 at 37°C	Decreased membrane fluidity with the potency being indomethacin > ibuprofen	[18]
Ibuprofen stereoisomers	Neuro-mimetic liposomal membranes prepared with POPC, POPE, POPS, POPI and SM (25 : 16 : 3 : 3 : 3 in molar ratio) and cholesterol (40 mol%)	200 μM	In HEPES buffer of pH 7.4 at 37°C	Decreased membrane fluidity with the potency being *S*(+)-ibuprofen > racemic ibuprofen > *R*(–)-ibuprofen	[19]
Piroxicam Meloxicam	DMPC small unilamellar vesicles	30 μM Drug : DMPC = 0.0008-0.12 : 1 in molar ratio	In MOPS buffer of pH 7.4 In glycine-HCl buffer of pH 2.0	Decreased membrane fluidity depending on a decrease of the molar ratio of drug to lipid Meloxicam was more effective in decreasing membrane fluidity at pH 2.0 than at pH 7.4	[20]
Celecoxib	DSPC multilamellar vesicles	1-24 mol% relative to DSPC	In PBS of pH 7.4	Increased membrane order at ≤6 mol% Decreased membrane order at ≥18 mol%	[21]
Celecoxib	Multilamellar vesicles prepared with DSPC plus cholesterol (3 : 1 in molar ratio)	6-18 mol% relative to DSPC	In PBS of pH 7.4 at 30°C and 65°C	Decreased membrane fluidity at 6 mol% in the gel phase Increased membrane fluidity at 18 mol% in the gel phase and in the liquid crystalline phase	[22]
Indomethacin	Multilamellar vesicles prepared with	Drug : EYPC/EYPA =	In PBS of pH 7.4 at 37°C	Increased both liposomal and cellular	[23]

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
Piroxicam	EYPC and EYPA (1 : 0.11 in molar ratio) Human neutrophils (5 x 10^6 cells/mL)	0.003-0.006 : 1 in molar ratio 50-100 μM		membrane microviscosity Piroxicam was more effective than indomethacin	
Diclofenac	Isolated unsealed human erythrocyte membranes suspended in phosphate buffer of pH 7.4 Large unilamellar vesicles prepared with DMPC (0.4 mM) to be suspended in water	10 μM to 2 mM	At 37°C and 18°C	Decreased erythrocyte membrane fluidity Decreased membrane fluidity at ~10 μM in the liquid crystalline phase but increased at 1-2 mM in the gel phase	[24]
Celecoxib	COX-2 expressing and non-expressing human colon cancer cells (5 x 10^8 cells/mL or 5 x 10^6 cells/mL)	20-70 μM treated for 24 hr	In PBS of pH 7.4	Acted on more fluid metastatic cells and decreased cellular membrane fluidity	[25]
Licofelone	Human colon cancer cells (5 x 10^5 cells/mL)	150 μM	In PBS of pH 7.4	Decreased cellular membrane fluidity	[26]
Celecoxib	Crude membranes from mouse neuroblastoma cells	Micromolar concentration	In imidazole buffer of pH 7.4 at 25°C	Decreased cellular membrane fluidity	[27]
Aspirin	Platelet suspensions (50 μg protein/mL) prepared from healthy human subjects who received aspirin for seven days	250 mg/day (p.o.)	At 37°C	Decreased platelet membrane fluidity in female subjects	[28]
Diclofenac	Colonic epithelial cells (1 x 10^6 cells/mL) isolated from rats that received carcinogen DMH (30 mg/kg, s.c.) weekly and diclofenac daily for six weeks	8 mg/kg (p.o.)	In PBS of pH 7.4 at 37°C	Counteracted the effect of DMH to increase cellular membrane fluidity	[29]
Celecoxib	Brush border membranes isolated from proximal and distal portions of the colon of rats that received carcinogen DMH (30 mg/kg, s.c.) weekly and celecoxib daily for six weeks	6 mg/kg (p.o.)	In maleate buffer of pH 6.5-6.8	Counteracted the effect of DMH to decrease cellular membrane microviscosity	[30]
Etoricoxib	Brush border membranes isolated from the jejunum segment of rats that received carcinogen DMH (30 mg/kg, s.c.) weekly and etoricoxib daily for six weeks	0.64 mg/kg (p.o.)	In EDTA-saline of pH 7.0	Inhibited the effect of DMH to decrease cellular membrane microviscosity Increased the activities of several intestinal membrane-associated enzymes that were decreased by DMH	[31]
Aspirin Celecoxib Etoricoxib	Liposomal membranes prepared with lipid extracts from colonic brush border membranes isolated from rats that received carcinogen DMH (30 mg/kg,	Aspirin: 60 mg/kg (p.o.) Celecoxib: 6 mg/kg (p.o.) Etoricoxib: 0.6 mg/kg (p.o.)	In Tris/HCl buffer of pH 7.4 at 37°C	Counteracted the effect of DMH to increase membrane fluidity with the potency being etoricoxib > celecoxib > aspirin	[32]

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
	s.c.) weekly and NSAIDs daily for six weeks				
Indomethacin Naproxen	Liposomal membranes prepared with gastric surface-active phospholipids extracted from the scraped surfaces of the oxyntic region of rat stomach DPPC unilamellar vesicles	Indomethacin: 2.2 mM Naproxen: 5.2 mM Drug : DPPC = 1 : 1 in molar ratio	In PBS of pH 7.4 at 25°C	Decreased membrane fluidity Elevated *T*m of membrane DPPC	[34]
Ibuprofen	Large unilamellar vesicles prepared with DMPC (0.4 mM) Human erythrocyte membranes (0.25 mg protein/mL) Liposomal membranes prepared with DMPC (5.6 mM) or DMPE (5.6 mM)	10-500 μM Ibuprofen : DMPC = 0.025-1.25 : 1 in molar ratio 10-500 μM 0.56-11.2 mM Ibuprofen : DMPC or DMPE = 0.1-2 : 1 in molar ratio	In PBS of pH 7.4 at 18°C and 37°C	Decreased membrane fluidity in the liquid crystalline phase more significantly than in the gel phase Increased erythrocyte membrane fluidity slightly in the liquid crystalline phase Reduced *T*m of membrane DMPC and DMPE	[35]
Indomethacin	DPPC large unilamellar vesicles	10-60 μM	In acetate buffer of pH 5.0 at 37°C HEPES buffer of pH 7.4 at 37°C	Increased membrane fluidity at pH 5.0 more significantly than at pH 7.4	[36]
Mefenamic acid	Multilamellar vesicles prepared with DMPC or DMPE	Mefenamic acid : phospholipid = 0.5-1 : 1 in molar ratio	In HEPES buffer of pH 7.4	Increased membrane fluidity below and above *T*m of membrane DMPC and DMPE	[37]
Tenoxicam Piroxicam Indomethacin Clonixin	Mouse splenocytes (1 x 10^6 cells/mL)	~650 μM	In HEPES buffer of pH 7.4 at 37°C	Increased cellular membrane fluidity with IC_{50} of 22.4 μM for tenoxicam, 27.0 μM for piroxicam, 88.9 μM for indomethacin and 291 μM for clonixin	[38]
Indomethacin	Rat gastric epithelial cells (1 x 10^5 cells/mL) cultured with indomethacin for 1-48 hr	0.1-1 mM	In a mixture of Dulbecco's modified Eagle medium and Ham's F-12 medium of pH ~7.4 at 37°	Increased cellular membrane fluidity after culturing cells at 0.1-1 mM for 1-3 hr Decreased cellular membrane fluidity after culturing cells at 0.3-1 mM for 48 hr	[39]
Lornoxicam Meloxicam Nimesulide	Large unilamellar vesicles prepared with EYPC (0.5 mM) or EYPC plus 30 mol% cholesterol Human leukemia monocytes (1 x 10^6 cells/mL), granulocytes (1 x 10^6 cells/mL) and mononuclear cells (1 x 10^6 cells/mL)	10-80 μM Drug : EYPC = 0.02-0.16 : 1 in molar ratio	In HEPES buffer of pH 7.4 at 37°C	Increased fluidity of both liposomal and cellular membranes with the potency being lornoxicam > meloxicam > nimesulide	[40]

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
	Mouse splenocytes (1 x 10^6 cells/mL) and macrophages (1 x 10^6 cells/mL)				
Aspirin Nimesulide Celecoxib	Brush border membranes isolated from different intestinal segments after administration of NSAIDs to rats for 28 days	40 mg/kg (p.o.)	In maleate buffer of pH 6.5-6.8 at 37°C	Increased fluidity of brush border membranes from duodenum and colon with the potency being nimesulide > aspirin > celecoxib	[41]
Tolmetin	Large unilamellar vesicles prepared with DPPC (0.5 mM)	40 μM Tolmetin : DPPC = 0.08 : 1 in molar ratio	In acetate buffer of pH 5.0 In HEPES buffer of pH 7.4	Interacted with membranes depending on pH and lipid phase to increase membrane fluidity and reduce *T*m of membrane DPPC	[42]
Naproxen	Liposomal membranes prepared with DMPC (5.6 mM) or DMPE (5.6 mM)	Naproxen : DMPC or DMPE = 0.1-2 : 1 in molar ratio	In PBS of pH 7.4	Reduced *T*m of membrane DMPC Showed less pronounced effect on DMPE membranes	[43]
Celecoxib	Large unilamellar vesicles prepared with DMPC (0.5 mM)	20 μM Celecoxib : DMPC = 0.04 : 1 in molar ratio	In acetate buffer of pH 5.0 In HEPES buffer of pH 7.4	Reduced *T*m of membrane DMPC at pH 7.4 more significantly than at pH 5.0	[44]
Piroxicam	DPPC bilayer liposomal membranes	Piroxicam : DPPC = 0.06-0.12 : 1 in molar ratio	In Tris-EDTA buffer of pH 7.4	Reduced *T*m of membrane DPPC	[45]
Indomethacin Nimesulide	DPPC multilamellar vesicles	Drug : DPPC = 0.1-0.4 : 1 in molar ratio	In HEPES buffer of pH 7.4	Reduced *T*m of membrane DPPC with the potency being indomethacin > nimesulide	[46]
Diclofenac Naproxen Ibuprofen	Liposomal membranes prepared with DMPC (5.6 mM)	Drug : DMPC = 0.1-2: 1 in molar ratio	In PBS of pH 7.4	Reduced *T*m of membrane DMPC with the potency being diclofenac > naproxen > ibuprofen	[47]
Diclofenac Naproxen Ibuprofen	DMPE bilayer liposomal membranes	Drug : DMPE = 0.1-2 : 1 in molar ratio	In PBS of pH 7.4	Reduced *T*m of membrane DMPE with the greatest potency of diclofenac	[48]
Diclofenac Ibuprofen Naproxen	DMPC bilayer liposomal membranes	Drug : DMPC = 0.1-2: 1 in molar ratio	In PBS of pH 7.4	Reduced *T*m of membrane DMPC with the greatest potency of diclofenac	[49]
Meloxicam Indomethacin Tolmetin Piroxicam	DPPC bilayer liposomal membranes	Drug : DPPC = 0.2 : 1 in molar ratio	In HEPES buffer of pH 7.4	Reduced *T*m of membrane DPPC with the potency being meloxicam > indomethacin > tolmetin > piroxicam	[50]
Tenoxicam Piroxicam Lornoxicam	DPPC bilayer liposomal membranes	Drug : DPPC = 0.01-0.20 : 1 in molar ratio Meloxicam : DPPC =	At pH 2.5 and 7.0	Reduced *T*m of membrane DPPC Elevated *T*m of membrane DPPC Reduced *T*m of membrane DPPC	[51]

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
Meloxicam		0.01 : 1 in molar ratio Meloxicam : DPPC = 0.05-0.20 : 1 in molar ratio			
Indomethacin Nimesulide	Multilamellar vesicles prepared with DMPC (0.05-1 mM)	Indomethacin: 40 μM Nimesulide: 50 μM Drug : DMPC = 0.05-0.90 : 1 in molar ratio	In HEPES buffer of pH 7.4	Reduced *T*m of membrane DMPC	[52]
Ibuprofen	Small unilamellar vesicles prepared with DMPC (~15 mM)	Ibuprofen : DMPC = 0.07 : 1 in molar ratio	At pH 1.5-8	Reduced *T*m of membrane DMPC at pH 2-8	[53]
Nimesulide Indomethacin Meloxicam Piroxicam	Unilamellar vesicles prepared with DPPC (0.5 mM)	40 μM Drug : DPPC = 0.08 : 1 in molar ratio	In acetate buffer of pH 5.0 In HEPES buffer of pH 7.4	Reduced *T*m of membrane DPPC with the potency being nimesulide > indomethacin > meloxicam > piroxicam	[54]
Diclofenac	Large unilamellar vesicles prepared with DOPC, DOPE and CL (1 : 1 : 1 in molar ratio, total lipids of ~1.5 mM) Large unilamellar vesicles prepared with DMPC (1.5 mM) Calcein-loaded large unilamellar vesicles prepared with DOPC, DOPE and CL (1 : 1 : 1 in molar ratio, total lipids of ~1.5 mM)	~80 μM Diclofenac : lipid = ~0.053 : 1 in molar ratio 40 μM Diclofenac : DMPC = 0.027 : 1 in molar ratio ~80 μM Diclofenac : lipid = ~0.053 : 1 in molar ratio	In HEPES buffer of pH 7.4 at 25°C In acetate buffer of pH 5.0 In HEPES buffer of pH 7.4 at 25°C	Increased membrane fluidity Reduced *T*m of membrane DMPC Increased membrane permeability	[55]
Meloxicam Piroxicam Tenoxicam	Small unilamellar vesicles prepared with DMPC (1 mM) or DMPC plus cholesterol (8 mol% relative to DMPC) Tb/dipicolinic acid co-encapsulated small unilamellar vesicles prepared with DMPC (0.7 mM) plus cholesterol (1-8 mol% relative to DMPC)	30 μM Drug : DMPC = 0.03 : 1 in molar ratio 30 μM Drug : DMPC = 0.043 : 1 in molar ratio	In MOPS buffer of pH 7.4 In TES buffer of pH 7.4 at 39 °C	Reduced *T*m of membrane DMPC with the potency being meloxicam > piroxicam > tenoxicam Increased membrane permeability with the potency being meloxicam > piroxicam > tenoxicam depending on an increase of cholesterol composition	[57]
Celecoxib Indomethacin Diclofenac Flufenamic acid Mefenamic acid Flurbiprofen	Calcein-loaded EYPC liposomes	0.01-100 mM Drug : EYPC = >0.33 : 1 in molar ratio	In phosphate buffer of pH 7.4 at 30°C	Increased membrane permeability with the potency being celecoxib > indomethacin > diclofenac > flufenamic acid > mefenamic acid > flurbiprofen > nimesulide > etodolac > ibuprofen > ketoprofen	[58]

NSAIDs	Membranes	Drug concentrations	Conditions	Induced effects	References
Nimesulide Etodolac Ibuprofen Ketoprofen					
Celecoxib	Calcein-loaded POPC large unilamellar vesicles Human erythrocytes	50-200 μM	Calcein release in phosphate buffer of pH 7.4 at 25°C Hemolysis in PBS of pH 7.4 at 37°C	Increased permeability of both liposomal and erythrocyte membranes	[59]

reflects its mobility in membrane lipid environments. Representative fluorophores used as a probe for FP and FA are 1,6-diphenyl-1,3,5-hexatriene (DPH), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH), 2-(9-anthroyloxy)stearic acid (2-AS), 6-(9-anthroyloxy)stearic acid (6-AS), 9-(9-anthroyloxy)stearic acid (9-AS), 12-(9-anthroyloxy)stearic acid (12-AS), 16-(9-anthroyloxy)palmitic acid (16-AP) and laurdan. They locate in different membrane regions to indicate a fluidity change in the membrane region specific to each individual probe. Fluorescent probes are subject to the rotational restriction imparted by membrane rigidity or order. When drugs decrease membrane fluidity, the induced more rigid (ordered) membranes disturb the probe rotation to emit the absorbed light in all directions, resulting in an increase of FP. On the contrary, drug-induced more fluid (disordered) membranes facilitate the probe rotation to emit the absorbed light in all directions, resulting in a decrease of FP. While FA is given by a simpler equation, calculated FA and FP are mathematically related and easily interconverted. FP and FA are inversely proportional to membrane fluidity.

3.1 Membrane Interactions of NSAIDs

3.1.1 Membrane fluidity decrease and lipid phase transition temperature elevation

The interactions of NSAIDs with artificial membranes were demonstrated by a number of FP or FA studies [14-20] and FTIR spectroscopic studies [21,22]. FP measurements with DPH indicated that NSAIDs act on liposomes prepared with egg yolk phosphatidylcholine (EYPC) to decrease membrane fluidity with the potency being nimesulide > mefenamic acid > flufenamic acid > celecoxib at 100 μM for each [14]. These drugs interacted with liposomal membranes consisting of EYPC plus cholesterol (1 : 0.75 in molar ratio) and decreased their fluidity with the potency being celecoxib > mefenamic acid > flufenamic acid. In a series of experiments of Lúcio et al. [15,16], large unilamellar vesicles were prepared with EYPC (0.5 mM) to be suspended in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer of pH 7.4. When the vesicles were subjected to the reactions with ~180 μM nimesulide, ~600 μM indomethacin and ~600 μM tolmetin, FA measurements with 2-AS, 6-AS, 9-AS and 12-AS at 25°C revealed that nimesulide interacts with liposomal membranes to decrease their fluidity at ≥4 μM most potently, followed by indomethacin and tolmetin [15]. These membrane-interacting drugs induced the largest FA increases in 12-AS, suggesting that they act preferentially on the deeper region of membrane lipid bilayers. Diclofenac of 50-400 μM decreased membrane fluidity by acting on the membrane surface of EYPC liposomes as shown by the largest FA change in 2-AS [16]. In the following derivative spectrophotometry of EYPC unilamellar vesicles suspended in different buffers, diclofenac of a neutral form showed a much larger partition coefficient in glycine-HCl buffer of pH 3.0 compared with diclofenac of an ionized form in HEPES buffer of pH 7.4 and borate-NaOH buffer of pH 10.3. In other FP studies with DPH, ibuprofen and indomethacin acted on unilamellar vesicles prepared with 1,2-dioleoylphosphatidylcholine (DOPC) to be suspended in phosphate buffer of pH 6.7 [17], biomimetic membranes prepared with 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE), 1-palmitoyl-2-oleoylphosphatidylserine (POPS), sphingomyelin (SM) and cholesterol (36 : 22 : 3.5 : 3.5 : 35 in molar ratio) to be suspended in phosphate buffer of pH 7.4 [18] and neuro-mimetic membranes prepared with POPC, POPE, POPS, 1-palmitoyl-2-oleoylphosphatidylinositol (POPI) and SM (25 : 16:3:3:3 in molar ratio) plus 40 mol% cholesterol to be suspended in HEPES buffer of pH 7.4 [19], resulting in a decrease of membrane fluidity at 100-200 μM with the greater potency of indomethacin. By measuring FA using their natural fluorescence, piroxicam and meloxicam of 30 μM for each were found to decrease the membrane fluidity of small unilamellar vesicles that were prepared with 1,2-dimyristoylphosphatidylcholine (DMPC; 0.25-3.75 mM) to be suspended in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer of pH 7.4 and glycine-HCl buffer of pH 2.0 [20]. Both drugs concentration- and pH-dependently interacted with DMPC liposomal membranes so that their relative effects to decrease membrane fluidity were more significant with decreasing the molar ratio of drug to lipid from 0.12: 1 to 0.008: 1 and meloxicam decreased membrane fluidity at pH 2.0 more potently than at pH 7.4. In FTIR spectroscopic experiments, celecoxib also caused concentration-dependent interactions with liposomal membranes that were prepared with 1,2-distearoylphosphatidylcholine (DSPC; 180 mM) alone or DSPC plus cholesterol (3 : 1 in molar ratio) to be suspended in phosphate-buffered saline (PBS) of pH 7.4 [21]. Celecoxib decreased membrane fluidity at drug

concentrations being ≤6 mol% relative to DSPC, but increased at drug concentrations being ≥18 mol%. Regardless of the presence of cholesterol in DSPC membranes, celecoxib decreased membrane fluidity at a drug concentration of 6 mol% relative to DSPC at 30°C in the gel phase, whereas increased at a drug concentration of 18 mol% at 30°C in the gel phase and at 65°C in the liquid crystalline phase [22].

Human neutrophils (5 x 10^6 cells/mL) suspended in PBS of pH 7.4 and multilamellar vesicles prepared with EYPC and egg yolk phosphatidic acid (EYPA) (13.5:1.5 in molar ratio, total lipids of 15 mM) to be suspended in PBS of pH 7.4 were treated with piroxicam and indomethacin at 37°C, followed by measuring FP with TMA-DPH [23]. Both drugs were found to decrease the fluidity of cellular membranes at 50-100 μM and liposomal membranes at the molar ratio of drug to lipid being 0.003-0.006:1. Piroxicam was more effective on neutrophil membranes than indomethacin. By measuring FP with laurdan, Suwalsky et al. [24] revealed that diclofenac acts on human erythrocytes suspended in phosphate buffer of pH 7.4 at 37°C to decrease the fluidity of isolated unsealed erythrocyte membranes at 10 μM to 2 mM. They also prepared large unilamellar vesicles with DMPC (0.4 mM) to be suspended in water for investigating the membrane interactivity of diclofenac at 37°C and 18°C. Diclofenac decreased the fluidity of liposomal membranes at ~10 μM in the liquid crystalline phase, but increased at 1-2 mM in the gel phase. COX expressing and non-expressing human colon cancer cells (5 x 10^6 or 5 x 10^8 cells/mL) suspended in PBS of pH 7.4 were treated with celecoxib at 20-70 μM for 24 hr, followed by ESR spectroscopy using 16-doxylstearic acid spin label and attenuated total reflectance-FTIR spectroscopy [25]. Irrespective of the COX-expression status, celecoxib decreased the fluidity of cellular membranes together with inhibiting the proliferation of cancer cells. Pyrene fluorescence excimer formation indicated that 150 μM licofelone decreases the fluidity of cellular membranes by acting on human colon cancer cells (5 x 10^5 cells/mL) suspended in PBS of pH 7.4 for 24 hr [26]. In FA measurements with DPH, celecoxib of micromolar concentrations decreased the fluidity of crude membranes prepared from mouse neuroblastoma cells to be suspended in imidazole buffer of pH 7.4 [27].

Platelet suspensions (50 μg protein/mL) were prepared from healthy human subjects who orally received aspirin (250 mg/day) for seven days [28]. FP measurements with DPH at 37°C indicated that aspirin decreases the fluidity of platelet membranes from female subjects. Rats were weekly administered with 1,2-dimethylhydrazine (DMH; 30 mg/kg, s.c.) for six weeks to induce colon cancers together with or without daily administration of diclofenac (8 mg/kg, p.o.) [29]. Colonic epithelial cells (1 x 10^6 cells/mL) were isolated and suspended in PBS of pH 7.4 to measure FP with DPH. Carcinogen DMH increased the fluidity of cellular membranes of colonic cancer cells, although diclofenac inhibited such a membrane effect of DMH. In the similar cancer-induction experiments [30,31], rats weekly received DMH (30 mg/kg, s.c.) together with daily receiving celecoxib (6 mg/kg, p.o.) or etoricoxib (0.64 mg/kg, p.o.). After six weeks, brush border membranes were isolated from proximal and distal portions of the colon to be suspended in maleate buffer of pH 6.6-6.8 and from the jejunum segment to be suspended in buffered saline of pH 7.0, followed by pyrene fluorescence excimer formation analyses [30,31]. Celecoxib counteracted the fluidity-increasing effects of carcinogen DMH on brush border membranes from both colon portions [30]. Although DMH increased the fluidity of brush border membranes from the jejunum segment together with inhibiting the activities of membrane-associated alkaline phosphatase, sucrase, lactase and maltase, etoricoxib suppressed such an increase in membrane fluidity and simultaneously increased the activities of these enzymes [31]. After rats weekly received DMH (30 mg/kg, s.c.) together with daily receiving aspirin (60 mg/kg, p.o.), celecoxib (6 mg/kg, p.o.) or etoricoxib (0.6 mg/kg, p.o.) for six weeks, liposomal membranes were prepared with lipids extracted from colonic brush border membranes to be suspended in tris(hydroxymethyl)aminomethane/HCl (Tris/HCl) buffer of pH 7.4 [32]. FP measurements with DPH at 37°C showed that these drugs counteracted the effects of carcinogen DMH to increase membrane fluidity with the potency being etoricoxib > celecoxib > aspirin.

As a temperature is raised, membrane lipids undergo a phase transition from the ordered gel phase in which phospholipid hydrocarbon chains are fully extended and closely packed to the disordered liquid crystalline phase in which phospholipid hydrocarbon chains are randomly oriented and fluid. The fluidity of membranes in the liquid crystalline phase is higher than that in the gel phase [33]. The main phase transition

temperature (*T*m) of membrane lipid is defined as a lipid-characteristic melting temperature, at which the lipid physical state changes from a rigid solid-like state to a fluid liquid-like state. Giraud et al. [34] prepared small unilamellar vesicles with 1,2-dipalmitoylphosphatidylcholine (DPPC) to be suspended in PBS of pH 7.4 and treated them with indomethacin and naproxen at the molar ratio of drug to DPPC being 1:1, followed by measuring FA with DPH. Indomethacin and naproxen elevated the *T*m of membrane DPPC, suggesting that both drugs decrease membrane fluidity. They also prepared liposome suspensions in PBS of pH 7.4 with gastric surface-active phospholipids extracted from the scraped surfaces of the oxyntic region of rat stomach. Indomethacin and naproxen decreased the fluidity of liposomal membranes at 0.8 mg/mL (2.2 mM) and 1.2 mg/mL (5.2 mM), respectively. By measuring FA with DPH and FP with laurdan, Manrique-Moreno et al*.* [35] demonstrated that 10-500 μM ibuprofen decreases the membrane fluidity of large unilamellar vesicles prepared with DMPC (0.4 mM) at 37°C in the liquid crystalline phase more potently than at 18°C in the gel phase, but slightly increases the membrane fluidity of isolated unsealed human erythrocytes (0.25 mg protein/mL) suspended in PBS of pH 7.4 at 37°C. However, their DSC experiments showed that 0.56-11.2 mM ibuprofen reduces the *T*m of membrane lipid by acting on liposomes prepared with DMPC (5.6 mM) or 1,2-dipalmitoyl-phosphatidylethanolamine (DMPE; 5.6 mM) to be suspended in PBS of pH 7.4 at the molar ratio of drug to lipid being 0.1-2:1.

3.1.2 Membrane fluidity increase, lipid phase transition temperature reduction and permeability increase

Indomethacin was subjected at 10-60 μM to the reactions with DPPC large unilamellar vesicles suspended in HEPES buffer of pH 7.4 or acetate buffer of pH 5.0, followed by FA measurements with TMA-DPH at 37°C [36]. Indomethacin was found to increase the fluidity of DPPC liposomal membranes at pH 5.0 more potently than at pH 7.4. Multilamellar vesicles were prepared with DMPC or DMPE to be suspended in HEPES buffer of pH 7.4 [37]. FTIR spectroscopy indicated that mefenamic acid acts on such liposomes to increase membrane fluidity at the molar ratio to lipid being 0.5-1:1.

Mouse splenocytes (1 x 10^6 cells/mL) suspended in HEPES buffer of pH 7.4 were treated with ~120 μM tenoxicam, ~120 μM piroxicam, ~350 μM indomethacin and ~650 μM clonixin, followed by measuring FA with DPH at 37°C [38]. Tenoxicam, piroxicam, indomethacin and clonixin increased the fluidity of cellular membranes with IC_{50} of 22.4 μM, 27.0 μM, 88.9 μM and 291 μM, respectively. FA measurements with 5-dodecanoylaminofluorescein revealed that indomethacin acts on rat gastric epithelial cells (1 x 10^5 cells/mL) for 1-3 hr to increase the fluidity of cellular membranes at 0.1-1 mM, but acts for 48 hr to decrease at 0.3-1 mM [39]. The membrane effects of 10-80 μM NSAIDs were studied using large unilamellar vesicles prepared with EYPC (0.5 mM) or EYPC (0.5 mM) plus cholesterol (30 mol%) to be suspended in HEPES buffer of pH 7.4 and cell suspensions (1 x 10^6 cells/mL) in HEPES buffer of pH 7.4 such as human leukemia monocytes, human granulocytes, human mononuclear cells, mouse splenocytes and mouse macrophages [40]. When measuring FA with DPH at 37°C, the tested drugs were found to increase the fluidity of both liposomal and cellular membranes with the potency being lornoxicam > meloxicam > nimesulide. After rats received NSAIDs (40 mg/kg, p.o.) for 28 days, brush border membranes were isolated from different intestinal segments [41]. Pyrene fluorescence excimer formation showed that the administered drugs increase the fluidity of membranes from duodenum and colon with the potency being nimesulide > aspirin > celecoxib.

Nunes et al. [42] prepared large unilamellar vesicles with DPPC (0.5 mM) to be suspended in acetate buffer of pH 5.0 and HEPES buffer of pH 7.4, and then treated them with tolmetin at the molar ratio of drug to lipid being 0.08:1, followed by measuring FA with TMA-DPH. Tolmetin interacted with DPPC liposomal membranes to increase their fluidity and reduce the *T*m of membrane DPPC depending on the pH of used buffers. In their following derivative spectrophotometric analyses, tolmetin showed a larger partition coefficient in DPPC membranes at pH 5.0 than at pH 7.4, suggesting that its membrane interaction is more pronounced under acidic conditions. The similar membrane effects were reported for naproxen acting on DMPC liposomes suspended in PBS buffer of pH 7.4 at the molar ratio of drug to lipid being 0.1-2:1 [43], celecoxib acting on DMPC large unilamellar vesicles suspended in HEPES buffer of pH 7.4 and acetate buffer of pH 5.0 at the molar ratio of drug to lipid being 0.04:1 [44] and piroxicam acting on DPPC liposomes suspended in Tris-

EDTA buffer of pH 7.4 at the molar ratio of drug to lipid being 0.06-0.12:1 [45]. Naproxen strongly interacted with DMPC liposomal membranes, although its effect on DMPE liposomal membranes was less pronounced [43]. Celecoxib was located more deeply inside DMPC membranes at pH 5.0 but closely to the surfaces of DMPC membranes at pH 7.4, which may be related to a larger increase of membrane fluidity at pH 7.4 [44]. Other NSAIDs reduced the *T*m of membrane lipid by acting on DPPC multilamellar vesicles suspended in HEPES buffer of pH 7.4 with the potency being indomethacin > nimesulide at the molar ratio of drug to lipid being 0.1-0.4:1 [46], on DMPC and DMPE liposomes suspended in PBS buffer of pH 7.4 with the potency being diclofenac > naproxen > ibuprofen at the molar ratio of drug to lipid being 0.1-2:1 [47-49] and on DPPC liposomes suspended in HEPES buffer of pH 7.4 with the potency being meloxicam > indomethacin > tolmetin > piroxicam at the molar ratio of drug to lipid being 0.2:1 [50]. Tenoxicam, piroxicam, lornoxicam and meloxicam acted on DPPC liposomes at pH 2.5 and pH 7.0 to change the *T*m of membrane DPPC at the molar ratio of drug to lipid being ~0.2:1 [51]. Among these oxicam NSAIDs, meloxicam reduced the *T*m at relatively high concentrations (the molar ratio of drug to DPPC = 0.05-0.20:1), whereas elevated the *T*m at a lower concentration (the molar ratio of drug to DPPC = 0.01:1). By acting on DMPC multilamellar vesicles suspended in HEPES buffer of pH 7.4, indomethacin and nimesulide reduced the *T*m of membrane DMPC as a function of drug concentrations in membranes that were calculated using the partition coefficient determined by derivative spectrophotometry [52]. Ibuprofen acted on DMPC small unilamellar vesicles at the molar ratio to lipid being 0.07:1 to reduce the *T*m of membrane DMPC at pH 2-8 [53]. When the molar ratio of drug to lipid was 0.08:1, NSAIDs acted on DPPC unilamellar vesicles suspended in HEPES buffer of pH 7.4 and acetate buffer of pH 5.0 to reduce the *T*m of membrane DPPC with the potency being nimesulide > indomethacin > meloxicam > piroxicam [54].

Fernandes et al. [55] prepared large unilamellar vesicles with DOPC, 1,2-dioleoylphosphatidylethanolamine (DOPE) and cardiolipin (CL) (1:1:1 in molar ratio, total lipids of ~1.5 mM) to be suspended in HEPES buffer of pH 7.4, and then treated them with diclofenac at ~80 μM. FA measurements with DPH and TMA-DPH revealed that diclofenac decreases membrane microviscosity by acting on the polar region of membrane lipid bilayers. In their following DSC experiment, 40 μM diclofenac acted on large unilamellar vesicles prepared with DMPC (1.5 mM) to be suspended in acetate buffer of pH 5.0, resulting in reduction of the *T*m of membrane lipid. They also investigated the effect of ~80 μM diclofenac on calcein-loaded large unilamellar vesicles that were prepared with DOPC, DOPE and CL (1:1:1 in molar ratio, total lipids of ~1.5 mM) to be suspended in HEPES buffer of pH 7.4. The calcein leakage analysis showed that diclofenac increases membrane permeability at the molar ratio of drug to lipid being ~0.053:1. An increase of membrane permeability is related to that of membrane fluidity [56]. Roy et al. [57] prepared small unilamellar vesicles with 1 mM DMPC or DMPC plus 8 mol% cholesterol to be suspended in MOPS buffer of pH 7.4 and treated them with oxicam NSAIDs at 30 μM, followed by DSC analysis. All the tested drugs acted on both vesicles to reduce the *T*m of membrane DMPC with the potency being meloxicam > piroxicam > tenoxicam. They also prepared Tb/dipicolinic acid co-encapsulated small unilamellar vesicles with 0.7 mM DMPC plus 1-8 mol% cholesterol to be suspended in 2-[tris(hydroxymethyl)methyl-amine]-1-ethanesulfonic acid (TES) buffer of pH 7.4 and analyzed the fluorescence intensity decrease after treating the vesicles with 30 μM oxicam NSAIDs at 39°C. The drugs increased membrane permeability with the potency being meloxicam > piroxicam > tenoxicam, correlating to the relative potency to reduce the *T*m of membrane DMPC. Tanaka et al. [58] compared the effects of 0.01-100 mM NSAIDs on calcein-loaded liposomal membranes that were prepared with 2 mM EYPC to be suspended in phosphate buffer of pH 7.4. When the molar ratio of drug to EYPC was >0.33:1, the drug-induced calcein leakage showed that all the tested drugs increase membrane permeability with the potency being celecoxib > indomethacin > diclofenac > flufenamic acid > mefenamic acid > flurbiprofen > nimesulide > etodolac > ibuprofen > ketoprofen when comparing the concentrations required for 20% release of calcein. In calcein leakage and hemolytic experiments, 50-200 μM celecoxib acted on POPC large unilamellar vesicles suspended in phosphate buffer of pH 7.4 and human erythrocytes suspended in PBS of pH 7.4, increasing membrane permeability of both liposomes and erythrocytes [59].

3.2 Interpretation of Membrane Interactivity

3.2.1 Membrane interactivity depending on drug concentration

When piroxicam and meloxicam act on DMPC liposomes, their relative effects to decrease membrane fluidity are more significant with decreasing the molar ratio of drug to lipid [20]. Piroxicam and indomethacin act on EYPC and EYPA multilamellar vesicles to decrease membrane fluidity at concentrations of drug relative to lipid being 0.003-0.006 in molar ratio [23]. In contrast to such membrane interactivity at low concentrations, mefenamic acid increases the fluidity of DMPC or DMPE liposomal membranes at the molar ratio of drug to lipid being 0.5-1:1 [37]. Diclofenac acts on DMPC unilamellar vesicles to decrease membrane fluidity at ~10 μM, but increase at 1-2 mM [24]. Despite acting on the same DSPC multilamellar vesicles, celecoxib decreases membrane fluidity at concentrations lower than 6 mol% relative to DSPC, whereas increases at concentrations higher than 18 mol% [21]. Meloxicam interacts with DPPC liposomal membranes to elevate the *T*m of membrane lipid at the molar ratio of drug to lipid being 0.01:1 [51]. At the molar ratio of drug to lipid being 0.1-2:1, however, not only meloxicam but also ibuprofen, naproxen, indomethacin, nimesulide, diclofenac, tolmetin and piroxicam reduce the *T*m of membrane lipid by interacting with phospholipid liposomal membranes [35,43,46-49]. Celecoxib, indomethacin, diclofenac, flufenamic acid, mefenamic acid, flurbiprofen, nimesulide, etodolac, ibuprofen and ketoprofen also increase the membrane permeability of EYPC liposomes at the molar ratio of drug to lipid being larger than 0.33:1 [58]. Ibuprofen concentration-dependently interacts with POPC liposomal membranes at 10-300 μM [60] and its interactivity with DMPC liposomal membranes at a concentration of 2 mol% relative to DMPC differs from that at higher concentrations of 10-20 mol% [61].

Celecoxib acts on human colon cancer cells to decrease the fluidity of cellular membranes at 20-70 μM [25]. Licofelon is effective at 150 μM in decreasing the membrane fluidity of human colon cancer cells [26]. However, tenoxicam, piroxicam, indomethacin and clonixin increase the membrane fluidity of mouse splenocytes at ~650 μM [38] and indomethacin increases the membrane fluidity of rat gastric epithelial cells at ~1 mM [39].

Orally administered diclofenac, celecoxib and etoricoxib decrease the fluidity of colonic epithelial cell membranes and colonic brush border membranes of rats, thereby inhibiting the fluidity-increasing effects of carcinogen DMH on cellular membranes [29-31]. On the other hand, higher-dose administrations of nimesulide, aspirin and celecoxib to rats conversely increase the fluidity of brush border membranes [41].

NSAIDs are likely to show a biphasic membrane effect to decrease membrane fluidity at relatively low concentrations but increase membrane fluidity at relatively high concentrations.

3.2.2 Membrane interactivity depending on pH

Since most NSAIDs have negatively chargeable groups, their p*K*a values range from 3 to 5.5 [62,63]. They are present in a neutral form at pH being < p*K*a, and in an anionic form at pH being > p*K*a. Therefore, acidic pH is preferable for NSAIDs to penetrate into membrane lipid bilayers by interacting with the acyl chains of phospholipids hydrophobically, whereas neutral or higher pH promotes the electrostatic interactions of NSAIDs with the polar head groups of phospholipids.

Meloxicam decreases the fluidity of DMPC liposomal membranes at pH 2.0 more potently than at pH 7.4 [20]. The effect of indomethacin to increase membrane fluidity is greater at pH 5.0 than at pH 7.4 [36].

The membrane interactivity of NSAIDs is likely to depend on the pH of interaction media. According to their p*K*a, NSAIDs are considered to modify the physicochemical membrane properties more significantly under acidic conditions.

3.2.3 Membrane interactivity depending on membrane lipid composition

Cellular membranes vary in lipid components and their composition, which differentially modulate the membrane interactivity of drugs. A specific lipid component also influences the drug distribution in lipid bilayers as reported for the presence of 20 mol% cholesterol in DMPC multilamellar vesicles that expels ibuprofen from the hydrophobic membrane core to locate it in the phospholipid head group region of membranes [61].

Indomethacin and mefenamic acid interact with phospholipid liposomal membranes to increase

their fluidity [36,37]. Lornoxicam, meloxicam and nimesulide increase membrane fluidity of EYPC unilamellar vesicles [40]. Tolmetin acts on DPPC unilamellar vesicles to not only increase membrane fluidity but also reduce the *T*m of membrane lipid [42]. The similar effects on phospholipid bilayer membranes are evident in naproxen [43], celecoxib [44], indomethacin [46,50], nimesulide [46,52,54], ibuprofen [47-49,53], diclofenac [47-49,55], tolmetin [50] and oxicam NSAIDs [51,57]. Celecoxib, indomethacin, diclofenac, flufenamic acid, mefenamic acid, flurbiprofen, nimesulide, etodolac, ibuprofen and ketoprofen also increase the permeability of EYPC liposomal membranes [58].

Biomembranes are composed of different phospholipid species and steroids. Ibuprofen and indomethacin interact with biomimetic membranes consisting of several phospholipids plus cholesterol to decrease membrane fluidity [18,19,21]. Piroxicam and indomethacin act on human neutrophils to decrease the fluidity of cellular membranes at relatively low concentrations [23]. Diclofenac also decreases the fluidity of erythrocyte membranes by acting on isolated unsealed human erythrocytes [24]. Celecoxib and licofelone are effective in decreasing the fluidity of cellular membranes of human colon cancer cells [25,26] and mouse neuroblastoma cells [27] as well as aspirin and diclofenac decrease the membrane fluidity of human platelets [28] and rat colonic epithelial cells [29]. Celecoxib and etoricoxib orally administered to rats decrease the fluidity of colonic brush border membranes [30,31], which are composed of phospholipids and cholesterol [64].

The membrane interactivity of NSAIDs depends on membrane lipid composition. NSAIDs are likely to decrease membrane fluidity when interacting with membranes composed of different phospholipids and cholesterol at relatively low concentrations, but increase membrane fluidity when interacting with membranes consisting of a single phospholipid component at relatively high concentrations.

3.3 Relevance to Anti-inflammatory Activity

NSAIDs have been experimentally studied with reference to their concentrations in blood or synovial fluid, which are estimated to be nanomolar levels after administration of the standard therapeutic doses [65,66]. However, NSAIDs are concentrated 10-30 times in inflamed tissues compared with their blood and synovial fluid concentrations [66,67]. The pH of inflamed tissues is lower (reduced to pH 5 or below) than the physiological pH of 7.4 [68,69]. Relevance of the membrane interactivity to the anti-inflammatory activity of NSAIDs should be discussed at micromolar concentrations under acidic conditions.

NSAIDs interact with liposomal membranes at acidic pH more potently than at pH 7.4 as reported for 30 μM meloxicam [20] and 10-60 μM indomethacin [36]. Nimesulide [15], ibuprofen and indomethacin [18], piroxicam and meloxicam [20], piroxicam and indomethacin [23], diclofenac [24], celecoxib [25] and licofelone [26] cause the membrane interactions at 4-150 μM to decrease membrane fluidity and elevate the *T*m of membrane lipid. NSAIDs are able to interact with lipid bilayer membranes at micromolar concentrations under inflammatory acidic conditions. A question arises as to how the membrane interactivity to decrease membrane fluidity is linked to COX inhibition.

Because integral membrane proteins are not rigid entities, their activities are regulated by the lipid environments surrounding them [70]. Sarcolemmal Na^+/K^+-ATPase in rat hearts is inhibited by increasing specific phospholipids and decreasing phospholipid side-chain arachidonic acid in sarcoplasmic reticulum membranes, suggesting a relation between the membrane-associated enzyme activity and the physicochemical membrane property [71]. A decrease of the fluidity of rat synaptosomal membranes correlates to that of the activity of membrane-bound Na^+/K^+-ATPase and Ca^{2+}-ATPase [72]. Decreasing membrane fluidity leads to inhibition of sarco(endo)plasmic Ca^{2+}-ATPase as this enzyme is inhibited by membrane fluidity-decreasing cholesterol and celecoxib [27]. The activity of adenylate cyclase is also suppressed by a decrease of membrane fluidity [73].

Membrane fluidity determines the activity of membrane-bound 5-lipoxygenase (5-LOX) that enzymatically converts arachidonic acid to leukotrienes to mediate inflammation. This cytoplasmatic enzyme is required to bind to nuclear membranes for activation [74]. 5-LOX effectively interacts with fluid membranes, but not with rigid membranes [9]. While COX is a

monotopic membrane protein localized in nuclear and endoplasmic reticulum membranes [75,76], this enzyme also interacts preferentially with fluid membranes to be activated as well as 5-LOX [9]. Membrane fluidity modulates the enzymatic activity of membrane-associated proteins that are activated by binding to the membrane domains with higher fluidity. The activities of membrane-associated COX and 5-LOX are speculated to be inhibited by a decrease of membrane fluidity, which can be induced by NSAIDs at relatively low concentrations under acidic conditions. Such speculative enzyme inhibition may be supported by the dual inhibitory effects of anti-inflammatory licofelone on COX and 5-LOX [77]. Licofelone modifies the structural organization of DPPC membranes [78] and decreases the fluidity of cellular membranes [26].

3.4 Relevance to Gastrointestinal Toxicity

NSAIDs potentially cause gastrointestinal inflammatory injury, erosion, ulceration and bleeding. Such toxicity has been explained by multifactorial mechanisms [79]. The most frequently cited mechanism is COX inhibition. COX possesses at least two isoforms: COX-1 and COX-2 with distinct enzymatic activity. COX-1 is constitutively expressed in most cell types especially as a predominant isoform in gastrointestinal tracts. This constitutive isozyme physiologically contributes to protecting the gastrointestinal mucosae and maintaining the gastrointestinal mucosal integrity. COX-2 is induced in inflamed tissues and over-expressed in neoplasms and solid tumors. Inducible COX-2 pathologically contributes to mediating the inflammatory reaction and promoting the cell proliferation. Therefore, COX-1 inhibition is theorized to produce adverse effects on gastrointestinal tracts by attenuating the physiological function, whereas COX-2 inhibition, to produce anti-inflammatory and anti-tumor effects by suppressing the inflammation and tumor cell growth [80]. However, there are some inconsistencies in gastrointestinal toxicity exhibition. Nonselective COX inhibitor indomethacin and selective COX-2 inhibitor celecoxib developed gastric and small intestinal ulcers in COX-1-deficient mice, while selective COX-1 inhibitor SC-560 did not induce intestinal ulceration in wild-type mice [81,82]. Dual inhibition of COX-1 and COX-2 caused the intestinal damage similar to that induced by indomethacin [82]. Patients underwent a capsule enteroscopy after a long-term oral ingestion of nonselective COX inhibitor ibuprofen, naproxen, indomethacin, ketoprofen or nabumetone, and selective COX-2 inhibitor celecoxib, etoricoxib, rofecoxib or valdecoxib [83]. Consequently, drug-induced intestinal damages were not different between nonselective COX inhibitors and selective COX-2 inhibitors. Human gastric lesions induced by NSAIDs did not correlate to their COX-2/COX-1 inhibition selectivity but well correlated to their p*K*a [84].

Orally administered NSAIDs reach the upper gastrointestinal tract, where gastric and duodenal mucosae are exposed to a large quantity of drugs. NSAIDs with p*K*a values of 3-6 are readily absorbed and transported to liver, thereafter being excreted into the bile, resulting in a repeated exposure of duodenal and jejunal mucosae to NSAIDs by enterohepatic circulation. The intraluminal pH in human stomach, duodenum and proximal jejunum are 1.5-3.0, 3.0-5.0 and 5.0-6.0, respectively [85]. The membrane interactions of NSAIDs in stomach, duodenum and upper small intestine should be discussed at high micromolar to low millimolar concentrations at acidic pH.

The mucosae of gastrointestinal tracts have the hydrophobic property to protect the underlying epithelia from gastric acid and luminal toxins. Such protective linings are mainly composed of phospholipids. Phosphatidylcholine (PC) is predominantly contained in gastric and duodenal mucosae of healthy subjects and patients with gastritis and duodenal ulcer as well as in those of dogs, rats and pigs, followed by phosphatidylethanolamine and phosphatidylinositol [86-88]. Of gastric mucosal PCs, the most abundant species are PC 16:0/18:1, PC 16:0/18:2, PC 16:0/20:4 and PC 18:0/20:4 [88,89].

The interactions of NSAIDs with phospholipids can be evaluated by NSAID-induced changes of the drug and phospholipid complex solubility in organic solvents and those of the physicochemical property of phospholipid membranes [90]. By using the latter methodology, indomethacin was proved to interact with DPPC liposomal membranes and increase their fluidity more potently at pH 5.0 than at pH 7.4 [36]. Mefenamic acid acts on DMPC and DMPE multilamellar vesicles to increase membrane fluidity at the molar ratio of drug to lipid being 0.5-1:1 [37]. Indomethacin is also effective in increasing the membrane fluidity of rat gastric epithelial cells at 0.1-1 mM [39]. In addition, naproxen [43], celecoxib [44] and

piroxicam [45] reduce the *T*m of membrane phospholipid by acting on DMPC, DMPE and DPPC liposomes. At the molar ratio of drug to lipid being 0.1-2:1, indomethacin, nimesulide, diclofenac, naproxen, ibuprofen, meloxicam, tolmetin, piroxicam and naproxen also reduce the *T*m of membrane DPPC, DMPC and DMPE [46-50]. Oral administration of nimesulide, aspirin and celecoxib (40 mg/kg for each) to rats increases the fluidity of intestinal brush border membranes [41].

NSAIDs are considered to interact with membranous phospholipids to change membrane fluidity and lipid phase transition with the resultant increase of gastrointestinal permeability to protons and toxins [91], thereby inhibiting the toxicity through impairment of the membrane-relevant biofunctions.

3.5 Relevance to Anti-tumor Activity

Different anti-tumor agents interact with membranes [92]. Tamoxifen used to treat estrogen receptor-positive breast cancer has the property to decrease the membrane fluidity of human cancer cells and liposomes at relatively low concentrations [93,94]. Antineoplastic doxorubicin acts on human myeloid leukemia cells to decrease the fluidity of plasma membranes [95] and this drug is also effective in decreasing the fluidity of liposomal membranes by preferentially acting on lipid bilayers in the more fluid phase [96]. Cisplatin and other platinum (II) analogs used as anti-cancer drugs for a wide range of tumors not only form the adducts with genomic DNA but also interact with plasma membranes to change the membrane organization and fluidity [97].

Celecoxib, ibuprofen, piroxicam, indomethacin and diclofenac act on biomimetic membranes and cellular membranes to decrease their fluidity [18,19,22,23,98]. Celecoxib decreases the membrane fluidity of human colon cancer cells at relatively low concentrations together with inhibiting their proliferation [25]. Although carcinogen DMH increases the membrane fluidity of rat colonic epithelial cells, diclofenac inhibits such an increase in membrane fluidity [29]. Celecoxib and etoricoxib also counteract the fluidity-increasing effects of carcinogen DMH on brush border membranes of rat colon and jejunum by decreasing membrane fluidity [30-32]. Anti-tumor chemicals with the COX-inhibitory activity similarly decrease the membrane fluidity of liposomes and tumor cells at micromolar concentrations [99-103].

The cell membrane dynamics are closely related to tumorigenesis-relevant enzyme activation, proliferative signal transduction, cell cycle progression and apoptosis induction. Tumor cells show higher membrane fluidity than their non-tumor counterparts [104]. Decreasing membrane fluidity leads to inhibition of the invasion and migration of cancer cells [105]. The modification of membrane fluidity is presumed to change the lipid environments optimal for the conformation of tumorigenesis-relevant proteins. NSAIDs to decrease the membrane fluidity would suppress a membrane fluidity increase occurring in tumor cells, thereby inhibiting their proliferation.

Membrane fluidity is also responsible for apoptosis induction by membrane-interacting tamoxifen [106] and flavonoids [102]. Bioactive compounds to decrease membrane fluidity and permeability can induce apoptosis in human breast cancer cells [107] and colon cancer cells [108]. While licofelone triggers apoptosis in human colon cancer cells independently from inhibition of COX and 5-LOX [109], this NSAID interacts with DPPC membranes [110]. Licofelone also inhibits the epidermal growth factor receptor signaling to induce the apoptosis of colon cancer cells by decreasing the fluidity of cellular membranes [26]. Baritaki et al. [111] recently published an excellent review about apoptosis and membrane fluidity.

Considering its relevance to the anti-tumor activity, the membrane interaction of NSAIDs may be one of possible strategies for cancer treatment and prevention as suggested by Alves et al. [92].

3.6 Relevance to Antimicrobial Activity

Ibuprofen of 31.3-250 μg produced inhibition zones of *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus brasiliensis* [1]. Ibuprofen also inhibited the growth of clinical isolates of *Staphylococcus aureus*, *Paracoccus yeei*, *Escherichia coli* and *Bacillus subtilis* with minimum inhibitory concentrations (MICs) of 6.1-12.1 mM [112]. Vedaprofen, bromfenac, carprofen, flufenamic acid and tolfenamic acid showed MICs ranging from 156 μM to 5 mM against *Bacillus subtilis*, *Staphylococcus aureus*, *Acinetobacter baylyi* and *Escherichia coli* [113]. Sulindac, indomethacin, ibuprofen and selective COX-2 inhibitor NS-398

were effective in inhibiting the growth of *Helicobacter pylori* (ATCC 49503 and ATCC 43504) to show minimum bactericidal concentrations of 103-470 μM, 175-349 μM, 1.2-2.4 mM and 0.1-1.1 mM, respectively [114]. Aspirin inhibited the growth of *Helicobacter pylori* at 1.1-2.2 mM together with increasing the susceptibility of *Helicobacter pylori* to antibiotic amoxycillin, clarithromycin and metronidazole [115]. Diclofenac, aspirin, indomethacin and ibuprofen not only inhibited the growth of *Escherichia coli* with MIC_{50} of 27 μM for diclofenac but also synergistically increased the effects of five antibiotic agents: amoxicillin, augmentin, cefotaxime, ciprofloxacin and gentamicin with different antibacterial mechanisms [116]. Such synergism between NSAIDs and antibiotics suggests that the mode of action different from conventional mechanisms (inhibition of cell wall synthesis, protein synthesis, nucleic acid synthesis and membrane function) may underlie the antimicrobial effects of NSAIDs.

Diclofenac acts on EYPC unilamellar vesicles at 50-400 μM [16] and DMPC unilamellar vesicles at as low concentrations as 10 μM [24], resulting in a decrease of membrane fluidity. Ibuprofen interacts with DOPC and DMPC liposomal membranes at 10-500 μM to decrease their fluidity [17,35]. Irrespective of the presence of cholesterol in membranes, ibuprofen and indomethacin induce a significant decrease in membrane fluidity at 50-200 μM [18,19,23]. NSAIDs exhibit the interactivity with microbe-mimetic membranes to decrease their fluidity at concentrations almost corresponding to MICs against different microbial species.

Antibacterial flavonoids show a positive correlation between the activity to decrease membrane fluidity and the activity to inhibit the growth of *Escherichia coli* [117]. Bactericidal peptide 3',6-dinonyl neamine decreases the membrane fluidity of *Pseudomonas aeruginosa* [118]. Antibacterial peptide cWFW acts on *Bacillus subtilis*, liposomes prepared with lipid extracts from *Escherichia coli* and liposomes consisting of POPE plus CL or 1-palmitoyl-2-oleoylphosphatidylglycerol to decrease all of their membrane fluidity [119]. While antibiotic amphotericin B and antifungal miconazole interact with fungal membranes to modify their permeability, both compounds decrease the fluidity of cellular membranes [120]. Non-polyene antibiotic primycin also interacts with the plasma membranes of *Candida albicans* to decrease their fluidity [121].

The membrane interaction to modify membrane fluidity is likely to mechanistically underlie the antimicrobial effects of NSAIDs as well as other membrane-interacting antibacterial and antifungal drugs.

3.7 Relevance to Cardiovascular Toxicity

While NSAIDs potentially induce myocardial infarction, heart attack, atrial fibrillation, myocardial ischemia and venous thrombosis, especially high doses of rofecoxib, celecoxib, parecoxib and valdecoxib increase the risk of cardiovascular complications [122]. Cardiovascular and thrombotic events are related to these selective COX-2 inhibition that disrupts a physiological balance between thromboxane A_2 and prostaglandin I_2 production. In addition, multifactorial mechanisms such as oxidative stress, production of reactive oxygen species (ROS), impairment of mitochondrial function and lipid peroxidation have been suggested for the cardiovascular toxicity of NSAIDs [123].

At supratherapeutic concentrations, bupivacaine and ropivacaine can cause myocardial infarction, atrial fibrillation and myocardial ischemia [124]. These local anesthetics interact with artificial and biological membranes to increase their fluidity at toxicologically-relevant concentrations with the potency correlating to relative cardiotoxicity [125-127]. Other cardiotoxic drugs also increase the fluidity of neuro-mimetic membranes [128-131]. Bupivacaine is localized at lipid-lipid or lipid-protein interfaces in biomembranes to increase membrane fluidity with the resultant functional interference of membrane-embedded sodium channels, which is referred to as one of mechanisms for its cardiotoxic effect [132].

NSAIDs similarly interact with membranes and modify their physicochemical properties at relatively high concentrations corresponding to cardiotoxic ones. Mefenamic acid relatively selective for COX-2 increases the membrane fluidity of phospholipid liposomes at the molar ratio of drug to lipid being 0.5-1:1 [37]. Oxicam NSAIDs relatively selective for COX-2 are also effective in increasing the fluidity of cellular membranes at ~0.65 mM [38]. The effects to reduce the *T*m of membrane lipid are produced by selective COX-2 inhibitor celecoxib [44], relatively selective COX-2 inhibitor diclofenac [47-49] and highly selective COX-2 inhibitor

meloxicam [50] at the molar ratio of drug to lipid being 0.1-2:1. Celecoxib also increases the membrane permeability of EYPC liposomes most potently, followed by nonselective COX inhibitors [58]. Selective COX-2 inhibitors are more closely associated with cardiovascular events than conventional NSAIDs [133].

Mitochondrial respiration and oxidative stress to increases ROS production are responsible for the cardiotoxicity of diclofenac, ibuprofen and naproxen [134]. Indomethacin, diclofenac, aspirin, nimesulide, meloxicam and piroxicam induce lipid peroxidation and cellular injury by impairing mitochondrial oxidative phosphorylation with the subsequent superoxide anion production independently from COX inhibition [135]. Compared with other tissues, heart is more significantly affected by drug-induced ROS production [124]. In bovine hearts, mitochondria are the main site of action for ROS [136]. Diclofenac causes the mitochondrial dysfunction in murine cardiomyocytes and hearts through excessive ROS production, which underlies its cardiotoxic effect [137]. While CL is predominantly located in mitochondrial inner membranes, diclofenac intensively interacts with CL-containing membranes to increase membrane fluidity [55] and membrane permeability [56].

3.8 COX Inhibition Selectivity and Drug Stereostructure Specificity

3.8.1 COX-2 selectivity and membrane interactivity

Nimesulide interacts with EYPC liposomal membranes to decrease their fluidity most potently, followed by mefenamic acid and flufenamic acid [14] and followed by indomethacin and tolmetin [15,16]. Such comparative membrane interactivity is related to the relative selectivity for COX-2 inhibition [122,138]. While NSAIDs decrease the fluidity of rat colonic brush border membranes with the potency being etoricoxib > celecoxib > aspirin [32], the order of such membrane interactivity is the same as that of inhibition selectivity for COX-2 [122].

When comparing at relatively high concentrations, NSAIDs increase the membrane fluidity of mouse splenocytes with the potency being tenoxicam > piroxicam > indomethacin > clonixin [39] and the fluidity of liposomal and cellular membranes with the potency being lornoxicam > meloxicam > nimesulide [40]. The relative membrane reactivity of these NSAIDs is consistent with the relative selectivity for COX-2 [122]. With respect to the effects on the phase transition of membrane DMPC, the order of intensity is diclofenac > naproxen > ibuprofen [47,48], diclofenac > naproxen > ibuprofen [49], and meloxicam > piroxicam > tenoxicam [57], which almost agrees with that of COX-2 inhibition selectivity. NSAIDs increase the membrane permeability of EYPC liposomes to show the relative potency being celecoxib > indomethacin > diclofenac > flufenamic acid > mefenamic acid > flurbiprofen > nimesulide > etodolac > ibuprofen > ketoprofen [58], which is consistent with that of COX-2 inhibition except indomethacin and nimesulide [122,138].

The membrane interactivity of NSAIDs is considered to correlate, at least partly, to their selectivity for COX-2 inhibition. The membrane interaction-associated enzyme inhibition different between COX-1 and COX-2 could induce selectivity for COX isozymes, resulting in an imbalance between thromboxane A_2 and prostaglandin I_2 production, which may cause thrombotic events or abnormal bleeding tendency. Thromboxane A_2 biosynthesized by the COX-1 catalyzing pathway is a vasoconstrictor or platelet aggregation facilitator, whereas prostaglandin I_2 biosynthesized by the COX-2 catalyzing pathway is a vasodilator or platelet aggregation preventer.

3.8.2 Stereostructure specificity and membrane interactivity

Chiral carbon-containing NSAIDs have non-superimposable mirror-image stereoisomers. Among them, ibuprofen, ketoprofen, loxoprofen, naproxen and etodolac are present as *S*(+)-enantiomer, *R*(–)-enantiomer or a racemic mixture of them. The *S*(+)-enantiomers are responsible for beneficial and adverse effects [139]. *S*(+)-Ibuprofen, *S*(+)-ketoprofen and *S*(+)-naproxen are more effective in inhibiting human platelet aggregation and suppressing human platelet prostaglandin production than *R*(–)-ibuprofen [140], *R*(–)-ketoprofen [141] and *R*(–)-naproxen [142], respectively.

Pharmacological and toxicological differences between enantiomers are interpreted by their discriminable spatial relation in the asymmetric environments of COX-constituting proteins that are entirely made up of only L-amino acids. In contrast to the interaction with membrane proteins, the interaction with membrane lipids

has been considered to lack stereostructure-specificity [141]. Since phospholipids and cholesterol are chirally pure to exist in only one enantiomer naturally, the membranes composed of them could act as a selector between enantiomeric drugs [143]. However, few studies have investigated the stereospecific membrane interaction of drugs except anesthetics [127] and terpenoids [144].

DPPC liposomes were found to recognize L-amino acids differentially from D-amino acids [145]. Okamoto et al. [17] suggested the possibility that phospholipid membranes may show the enantioselective adsorption to discriminate between *S*(+)-ibuprofen and *R*(–)-ibuprofen. Membrane lipid bilayers exhibit chirality depending on an increase of cholesterol composition and the absolute configuration of cholesterol influences the physicochemical membrane property [146]. Amphidinol 3 enantioselectively binds to POPC membranes containing specific sterols because this antifungal agent interacts with cholesterol with a 3β-hydroxyl group, but not with epicholesterol with a 3α-hydroxyl group [147].

When interacting with biomimetic membranes containing cholesterol, ibuprofen stereospecifically decreased membrane fluidity with the potency being *S*(+)-ibuprofen > racemic ibuprofen > *R*(–)-ibuprofen [18]. Ibuprofen also interacted with neuro-mimetic membranes consisting of 40 mol% cholesterol and 60 mol% phospholipids to decrease their fluidity with the relative potency being 1.90 ± 0.08, 1.22 ± 0.05 and 1.00 ± 0.02 for *S*(+)-ibuprofen, racemic ibuprofen and *R*(–)-ibuprofen, respectively, at 200 μM for each [19]. The relative membrane interactivity is estimated to be 1.90 for *S*(+)-ibuprofen/*R*(–)-ibuprofen and 1.56 for *S*(+)-ibuprofen/racemic ibuprofen. The relative activity to inhibit prostaglandin synthesis is 160 for *S*(+)-ibuprofen/*R*(–)-ibuprofen and 1.6-2.0 for *S*(+)-ibuprofen/racemic ibuprofen when compared using bovine seminal vesicle microsomal enzymes and human platelet enzymes, and the relative anti-inflammatory activity is 1.4 for *S*(+)-ibuprofen/*R*(–)-ibuprofen and 1.2-1.3 for *S*(+)-ibuprofen/racemic ibuprofen when orally administered to mice and rats [139].

4. CONCLUSION

Although lipids were previously considered as only a structural component in biomembranes, they can play a critical role in regulating the activity of membrane-associated proteins and maintaining the membrane-associated physiological conditions. In addition to enzyme proteins, NSAIDs structure-specifically act on membrane-constituting lipids to modify membrane fluidity, lipid phase transition and permeability depending on drug concentration, medium pH and membrane lipid composition, all of which are influential on the features and potencies of the membrane interactivity of NSAIDs. The interaction of NSAIDs with lipid bilayer membranes at relatively low concentrations to decrease membrane fluidity is responsible for their anti-inflammatory and anti-tumor activity by inhibiting COX indirectly through an alteration of the lipid environments surrounding membrane-associated enzyme proteins. The interaction of NSAIDs with membranous phospholipids at relatively high concentrations to increase membrane fluidity is responsible for their gastrointestinal and cardiovascular toxicity by impairing the membrane-relevant biofunctions. Other diverse effects of NSAIDs may also be related to their membrane interactions. Although not all NSAIDs interact with membranes and not all membrane-interacting drugs exhibit the beneficial activity or the adverse toxicity, the present review gives insights into one of possible modes of action of NSAIDs. The membrane interactivity will be available to study NSAIDs from a novel mechanistic point of view.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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