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REVIEW
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Interaction of drugs with lipid raft membrane domains as a possible target

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ABSTRACT

Introduction: Plasma membranes are not the homogeneous bilayers of uniformly distributed lipids but the lipid complex with laterally separated lipid raft membrane domains, which provide receptor, ion channel and enzyme proteins with a platform. The aim of this article is to review the mechanistic interaction of drugs with membrane lipid rafts and address the question whether drugs induce physicochemical changes in raft-constituting and raft-surrounding membranes.

Methods: Literature searches of PubMed/MEDLINE and Google Scholar databases from 2000 to 2020 were conducted to include articles published in English in internationally recognized journals. Collected articles were independently reviewed by title, abstract and text for relevance.

Results: The literature search indicated that pharmacologically diverse drugs interact with raft model membranes and cellular membrane lipid rafts. They could physicochemically modify functional protein-localizing membrane lipid rafts and the membranes surrounding such domains, affecting the raft organizational integrity with the resultant exhibition of pharmacological activity. Raft-acting drugs were characterized as ones to decrease membrane fluidity, induce liquid-ordered phase or order plasma membranes, leading to lipid raft formation; and ones to increase membrane fluidity, induce liquid-disordered phase or reduce phase transition temperature, leading to lipid raft disruption.

Conclusion: Targeting lipid raft membrane domains would open a new way for drug design and development. Since angiotensin-converting enzyme 2 receptors which are a cell-specific target of and responsible for the cellular entry of novel coronavirus are localized in lipid rafts, agents that specifically disrupt the relevant rafts may be a drug against coronavirus disease 2019.

Keywords: Drug target, Fluidity, Lipid raft, Membrane domain, Membrane interaction

Introduction

Since Singer and Nicolson proposed a fluid mosaic model, the concept of membrane organization has progressively changed, that is, plasma membranes are not the homogeneous bilayers of uniformly distributed lipids but the lipid complex with laterally separated membrane domains such as lipid rafts and caveolae (1). Lipid rafts are small (10-200 nm), heterogeneous, dynamic, and cholesterol- and sphingolipid-enriched membrane domains that are distinct from the rest of the membrane structures (2), whereas caveolae are a subset of lipid rafts and organizationally maintained by characteristic protein caveolins (3). Lipid rafts in a liquid-ordered (L_o) phase coexist with the bulk of membranes in a liquid-disordered (L_d) phase (4). Lipid raft membrane domains play an important role in cellular signal transduction and trafficking by compartmentalizing membranes and providing functional membrane proteins with a platform (4-7). Pharmacologically relevant receptors, ion channels and enzymes are localized or cluster in membrane lipid rafts and caveolae (8-11).

Given the localization of receptors, ion channels and enzymes in membrane lipid rafts, the mode of drug action is first interpretable in a simple manner of receptor/channel/enzyme and ligand interaction as known in the conventional mechanistic theory. The second possibility is that drugs may act on membrane lipids to affect the organizational integrity of lipid rafts, resulting in modulation of the activity of receptors, ion channels and enzymes embedded in membrane domains. It is of much interest to know whether drugs interact

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preferentially with lipid rafts compared with non-raft overall membrane lipid bilayers and whether such interaction at a membrane lipid level is linked to pharmacological and cytotoxic effects of drugs. While cholesterol is essential to raft and caveola formation, the regulatory effects of membrane domains on receptors and ion channels were confirmed by depleting cholesterol in plasma membranes (12-15).

The purpose of the present study is to review the interaction of drugs with membrane lipid rafts and the membranes surrounding such domains by searching scientific articles from a mechanistic point of view in order to gain new insights into a drug target. Since various proteins embedded in membranes are functionally modulated by membrane fluidity, order and phase transition, the focus of our review is on addressing the question whether drugs modify the physicochemical properties of raft-constituting and raft-surrounding membranes to affect the formation, stability and integrity of lipid raft membrane domains.

Methods

The present review is based on articles that were retrieved from PubMed/MEDLINE and Google Scholar by searching databases from 2000 to 2020. The publications earlier than 2000 were exceptionally cited if they are essential to advancing the discussion. Research papers published in English in internationally recognized journals and online journals were preferred, but review articles were additionally used to deepen understanding of the concept of plasma membranes and the mode of drug action. For reviewing as diverse drugs as possible without confining to a specific class of drug, the literature searches were carried out using the following terms or combinations thereof: “lipid raft,” “caveola,” “membrane domain,” “membrane interaction,” “fluidity,” “receptor,” “channel” and “enzyme.” Collected articles were independently reviewed by title, abstract and text for relevance with preference to more recent publications.

Results and discussion

Drug and raft interaction methodology

Since the methodology of drug and membrane raft interaction is essential to facilitate readers’ understanding of individual studies, representative experiments are mentioned as follows.

In in vitro experiments, drugs are subjected to the reaction with raft model (raft-like) membranes or liposomes that mimic the lipid composition and property of lipid raft micro domains (16,17). Ternary lipid membranes are used as a raft model, which is frequently prepared with an equimolar mixture of 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), sphingomyelin (SM) and cholesterol (18), in which cholesterol functions as a spacer between sphingolipid hydrocarbon chains and as a glue to keep the raft assembly together (19). Such raft model membranes have the advantage that the membrane effects of drugs can be determined more easily than in vivo experiments (20). Lipid rafts isolated from cells are also used experimentally. Since lipid rafts are relatively insoluble in cold non-ionic detergents, cells are treated with Triton X-100 and membrane lipid rafts are fractionated by sucrose density gradient centrifugation (SDGC) (21).

In in vivo experiments, human and animal subjects are treated with drugs, followed by SDGC to isolate cellular membrane lipid rafts. Cholesterol is not only a critical determinant for membrane fluidity but also an essential component to form the L_o membrane domains. Cellular cholesterol contents are manipulated by treating animals with cholesterol metabolic inhibitors, culturing cells in cholesterol-deficient media and using cholesterol-depleting agents. Methyl-β-cyclodextrin (MBC), to form a 2:1 complex with cholesterol (22), is most widely used for cholesterol depletion (23).

Drug-induced physicochemical or biophysical changes in raft model membranes and membrane lipid rafts are determined by fluorescence polarization (FP) or anisotropy (FA), differential scanning calorimetry (DSC), nuclear magnetic resonance (NMR) spectroscopy, neutron diffraction (ND), X-ray diffraction (XD) and their complementary combination.

General anesthetics

General anesthetics and their related sedatives, anxiolytics and adjuncts act on inhibitory γ-aminobutyric acid type A ($GABA_A$) receptors and excitatory *N*-methyl-D-aspartate (NMDA) receptors (24). Intravenous and inhalational anesthetics are a positive allosteric modulator or a direct activator of $GABA_A$ receptors to enhance their inhibitory functions, inducing general anesthesia, sedation, anxiolysis and convulsion cessation (25). Inhalational anesthetics are also a non-competitive antagonist of NMDA receptors to reduce neuronal excitation, producing analgesic, sedative and anesthesia-maintaining effects (26,27). These anesthesia-relevant $GABA_A$ receptors and NMDA receptors are associated with lipid raft membrane domains (28,29). Results of the literature search indicated that general anesthetics interact with membrane lipid rafts and membranes as shown in Table I.

Intravenous anesthetic propofol

FP experiments demonstrated that propofol structure-specifically interacts with binary liposomal membranes prepared with 80 mol% POPC and 20 mol% cholesterol (30) and quinary liposomal membranes prepared with 55 mol% phospholipids (POPC, SM, 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) and 1-palmitoyl-2-oleoylphosphatidylserine (POPS)) and 45 mol% cholesterol (31), resulting in an increase of membrane fluidity at clinically relevant 0.125-10 μM. L_o and L_d phase equilibrium is present in giant plasma membrane vesicles (GPMVs) isolated from rat basophil leukemia cells, which are used as a model of membrane heterogeneity for lipid rafts. Gray et al treated GPMVs with propofol and its structural analogs to examine their effects on liquid-liquid transition by analyzing the lateral distribution of fluorescent probe DiI-C_{12} microscopically (32). Propofol reduced the critical transition temperature at 2.5-10 μM, but not 2,6-di-*tert*-butylphenol without the anesthetic activity at the same concentrations. Therefore, propofol is considered

TABLE I - Interaction of general anesthetics with lipid raft membrane domains and membranes

Drug class	Drug	Membrane	Induced membrane modification	Reference
Intravenous anesthetic	Propofol (0.125-1.0 μM)	Binary liposomal membranes (80 mol% POPC and 20 mol% cholesterol)	Increased membrane fluidity	30
Intravenous anesthetic	Propofol (10 μM)	Quinary liposomal membranes (55 mol% phospholipids (POPC, SM, POPE and POPS) and 45 mol% cholesterol)	Increased membrane fluidity	31
Intravenous anesthetic	Propofol (2.5-10 μM)	GPMVs isolated from rat basophil leukemia cells	Reduced the critical transition temperature structure-specifically	32
Intravenous anesthetic	Propofol (10 and 30 μM)	Human airway smooth muscle cell membranes	Reduced the intracellular Ca^{2+} concentration responses to 10 μM histamine, disrupted caveolae and decreased caveolin-1 expression	33
Inhalational anesthetic	Isoflurane (1 and 5 mM)	POPC/cholesterol liposomal membranes, erythrocyte ghosts and brain endothelial cell-mimetic membranes	Increased membrane fluidity	34
Inhalational anesthetic	Isoflurane (2.5-12 mM)	LUVs (62.5 mol% DPPC and 37.5 mol% cholesterol)	Weakened the sterol-phospholipid association in cholesterol-rich L_o phase membranes	35
Inhalational anesthetic	Halothane (1.5 mol%)	Multilayer membranes (DPPC and DLPC, 1:1 molar ratio)	Reduced the transition temperature by about 5°C	36
Inhalational anesthetic	Xenon (4.6-fold MAC) Nitrous oxide (4.6-fold MAC) Halothane (three- to fivefold MAC) Isoflurane (three- to fivefold MAC)	Raft model membranes (DOPC, SM and cholesterol, 1:1:0.2 molar ratio)	Increased the L_d phase Decreased the relative intensity of L_o to L_d phase	37
Barbiturate	Rats injected with sodium pentobarbital (50 mg/kg, i.p.)	Lipid rafts isolated from rat brains 15 minutes after drug injection	Reduced the transition temperature	38

DLPC = 1,2-dilauroylphosphatidylcholine; DOPC = 1,2-dioleoylphosphatidylcholine; DPPC = 1,2-dipalmitoylphosphatidylcholine; GPMV = giant plasma membrane vesicle; LUV = large unilamellar vesicle; MAC = minimum alveolar concentration; POPC = 1-palmitoyl-2-oleoylphosphatidylcholine; POPE = 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPS = 1-palmitoyl-2-oleoylphosphatidylserine; SM = sphingomyelin.

to decrease the magnitude of membrane heterogeneity structure-specifically, affecting receptor and ion channel proteins sensitive to raft heterogeneity. While propofol is known to produce bronchodilatation, the airway relaxation involves a decrease of Ca^{2+} concentrations in airway smooth muscle cells that are regulated by caveolae. By exposing human airway smooth muscle cells to propofol at 10 and 30 μM, Grim et al found that propofol increases in membrane caveolae and reduces the intracellular Ca^{2+} concentration response to 10 μM histamine (33). They also suggested that propofol may induce caveolar disruption and caveolin-1 expression decrease.

Inhalational anesthetics

Patel et al investigated the membrane effects of isoflurane using different membrane systems such as POPC/cholesterol liposomal membranes, erythrocyte ghosts and brain endothelial cell-mimetic membranes (34). FA measurements indicated that isoflurane increases the membrane fluidity at 1 and 5 mM. Turkyilmaz et al prepared large unilamellar vesicles (LUVs) with 1,2-dipalmitoylphosphatidylcholine (DPPC) and cholesterol to be 2.5 mol% or 37.5 mol% cholesterol-containing DPPC membranes to verify the membrane effects of inhalational anesthetics (35). Isoflurane and halothane weakened and strengthened the sterol-phospholipid association in cholesterol-rich L_o phase membranes and in cholesterol-poor L_d phase membranes, respectively, at 2.5-12 mM. In ND and XD experiments of Weinrich et al, halothane was subjected to the reaction with multilayer membranes that were prepared with an equimolar mixture of DPPC and 1,2-dilauroylphosphatidylcholine (DLPC) to form distinct DPPC-rich ordered and DLPC-rich fluid phase (36). Halothane reduced the transition temperature by about 5°C at 1.5 mol% corresponding to about twice the minimum alveolar concentration (MAC) for human anesthesia, but not non-anesthetic

1,2-dichlorohexafluorocyclobutane even at fivefold MAC. Weinrich and Worcester determined the effects of different anesthetics on liquid phase distribution in raft model membranes prepared with 1,2-dioleoylphosphatidylcholine (DOPC), SM and cholesterol (1:1:0.2 molar ratio) by ND and XD analysis (37). Xenon and nitrous oxide increased the L_d phase at 4.6-fold MAC, and halothane and isoflurane decreased the relative intensity of L_o to L_d phase at three- to fivefold MAC.

Barbiturate

Pentobarbital is intravenously and intraperitoneally administered especially in veterinary anesthesia or sedation. Sierra-Valdez et al characterized the in vivo effects of pentobarbital on rat brain lipid rafts, which were isolated 15 min after injecting rats with sodium pentobarbital at 50 mg/kg intraperitoneally (38). DSC analysis revealed that pentobarbital reduces the transition temperature from L_o to L_d phase.

Membranous sodium channel blocker local anesthetics

Local anesthetics reversibly block voltage-gated sodium (Nav) channels that are responsible for the initiation and propagation of action potentials in excitable cells, inhibiting sensory and motor functions (39). Among nine distinct Nav channels (Nav1.1 to Nav1.9) cloned from mammals, Nav1.8 channel plays a crucial role in pain transmission and this isoform is implicated as a site of action for anesthetic and analgesic drugs. While Nav channels are present in caveolae-type and non-caveolae-type lipid rafts, Nav1.8 channel clustering in such membrane domains is essential to the propagation of action potentials in nociceptive axons (40,41). Nav1.8 channels are associated with lipid rafts in rat dorsal root ganglionic neurons, but cholesterol depletion induces dissociation between Nav1.8 channels and lipid rafts (42). Results of the literature search on the interaction of local anesthetics with membrane lipid rafts and membranes are shown in Table II.

Kamata et al incubated human erythrocytes with lidocaine at 18.4 mM and prepared erythrocyte ghosts, followed by SDGC fractionation and immunoblotting analysis for flotillin-1 (caveolae-associated integral membrane protein) that is assumed to stabilize lipid rafts (43). Lidocaine reversibly disrupted erythrocyte membrane lipid rafts and abolished flotillin-1 in lipid rafts together with depleting cholesterol. Bandeiras et al treated LUVs prepared with POPC, SM and cholesterol (1:1:1 molar ratio) with tetracaine and lidocaine, and then evaluated their membrane effects by DSC and phosphorus NMR spectroscopy (44). Tetracaine and lidocaine increased the fluidity of raft-like membranes at 25 and 69 mM,

TABLE II - Interaction of membranous sodium channel blocker local anesthetics with lipid raft membrane domains and membranes

Drug class	Drug	Membrane	Induced membrane modification	Reference
Local anesthetic	Lidocaine (18.4 mM)	Human erythrocyte membranes	Disrupted membrane rafts reversely and abolished flotillin-1 in lipid rafts	43
Local anesthetic	Tetracaine (25 mM) Lidocaine (69 mM)	LUVs (POPC, SM and cholesterol, 1:1:1 molar ratio)	Increased the fluidity of raft-like membranes	44
Local anesthetic	Dibucaine (0.05 and 0.2 mM)	Raft-like membranes (POPC, DPPC and cholesterol, 2:1:1 molar ratio)	Reduced the miscibility temperature of L_o and L_d phase separation	45
Local anesthetic	Lidocaine (10-20 mol%) Tetracaine (10-20 mol%)	Raft-like membranes (POPC, DPPC and cholesterol, 2:2:1 molar ratio)	Reduced the miscibility temperature of L_o and L_d phase separation and decreased the line tension at L_o/L_d phase boundary	46
Local anesthetic	Dibucaine (0.2 mM) Tetracaine (0.2 mM)	LUVs (POPC, SM and cholesterol, 16:43:41 molar ratio)	Increased the fluidity of L_o phase membranes, but not L_d phase membranes	47
Local anesthetic	Lidocaine (50-200 μM) Bupivacaine (50-200 μM) Ropivacaine (50-200 μM) Prilocaine (50-200 μM)	SUVs (DOPC, POPE, SM, CB and cholesterol, 16.7:16.7:16.7:16.7:33.3; DOPC, SM and cholesterol, 33.3:33.3:33.3; and DOPC, POPE, POPS, SM and cholesterol, 5:5:10:40:40 molar ratio)	Increased the membrane fluidity with the relative potency being bupivacaine > ropivacaine > lidocaine > prilocaine More effective in interacting with the reference biomimetic membranes than the raft model membranes	49
Local anesthetic	Bupivacaine enantiomers (5-50 μM)	SUVs (POPC, POPE, POPS, POPI, SM, cardiolipin and cholesterol, 25:16:3:3:3:10:40 molar ratio)	Increased the fluidity of biomimetic membranes with the relative potency being *R*(+)-bupivacaine > racemic bupivacaine > *S*(–)-bupivacaine	50

CB = cerebroside; DOPC = 1,2-dioleoylphosphatidylcholine; DPPC = 1,2-dipalmitoylphosphatidylcholine; LUV = large unilamellar vesicle; POPC = 1-palmitoyl-2-oleoylphosphatidylcholine; POPE = 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPI = 1-palmitoyl-2-oleoylphosphatidylinositol; POPS = 1-palmitoyl-2-oleoylphosphatidylserine; SM = sphingomyelin; SUV = small unilamellar vesicle.

respectively. Yoshida et al prepared lipid bilayer membranes with DOPC, DPPC and cholesterol (2:1:1 molar ratio) to be laterally separated into L_o and L_d phase together with labeling the membranes with fluorescent probe rhodamine DHPE (dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine) (45). After treating the membrane preparations with dibucaine at 0.05 and 0.2 mM, they observed the raft-like membrane domains by fluorescence microscopy at 20-40°C to determine changes in miscibility temperature of the L_o and L_d phase separation and in line tension at the L_o/L_d phase boundary. Dibucaine reduced the miscibility temperature, which was accompanied by the line tension decrease. Dibucaine also made the L_o domains smaller at 25°C, although most membranes were present without such raft-like domains at above 25°C. In a similar microscopic experiment using liposomes prepared with DOPC, DPPC and cholesterol (2:2:1 molar ratio), lidocaine and tetracaine reduced the miscibility temperature of ternary membranes at 10-20 mol% relative to liposomal lipids, but not binary membranes without cholesterol (46). Both local anesthetics also decreased the line tension at the L_o/L_d phase boundary. Kinoshita et al performed FA experiments to reveal the effects of local anesthetics on raft-like L_o/non-raft L_d phase membranes by using LUVs that were prepared with DOPC, SM and cholesterol (16:43:41 and 65:16:19 in molar ratio for L_o phase and L_d phase, respectively) (47). Dibucaine disordered the lipid packing or increased the fluidity of L_o phase membranes at 0.2 mM more potently than tetracaine, whereas dibucaine and tetracaine showed no significant effects on L_d phase membranes. However, these studies (43-47) used drug concentrations much higher than clinically and experimentally relevant ones (48) and the tested dibucaine and tetracaine are not widely used in clinical anesthesia.

Tsuchiya et al prepared small unilamellar vesicles (SUVs) with DOPC, POPE, SM, cerebroside (CB) and cholesterol (16.7:16.7:16.7:16.7:33.3 molar ratio); DOPC, SM and cholesterol (33.3:33.3:33.3 molar ratio); and DOPC, POPE, POPS, SM and cholesterol (5:5:10:40:40 molar ratio) for raft model membranes, and POPC, POPE, POPS, 1-palmitoyl-2-oleoylphosphatidylinositol (POPI), SM, cardiolipin and cholesterol (25:16:3:3:3:10:40 molar ratio) for reference biomimetic membranes (49,50). They treated these membrane preparations with lidocaine, bupivacaine, ropivacaine and prilocaine at anesthetic and cardiotoxic concentrations, followed by FP measurements. All the tested anesthetics interacted with raft model and biomimetic membranes to increase the membrane fluidity at 50-200 μM with the relative potency being bupivacaine > ropivacaine > lidocaine > prilocaine (49). They were more effective in interacting with the reference membranes than the raft membranes. Biomimetic membranes showed different interactivity with the relative potency being *R*(+)-bupivacaine > racemic bupivacaine > *S*(–)-bupivacaine at 5-50 μM, being consistent with the rank order of their anesthetic and cardiotoxic effects (50). However, raft model membranes did not exhibit significant enantioselectivity as the reference biomimetic membranes. These results may suggest that lipid rafts are less likely to contribute at least to the enantioselective effects of local anesthetics.

Membranous receptor- and enzyme-acting drugs

Results of the literature search on the interaction of receptor-acting adrenergic and opioid drugs and enzyme-acting anti-inflammatory drugs with membrane lipid rafts and membranes are shown in Table III.

Beta-adrenergic blockers

Beta-blockers are perioperatively used to reduce the risk of myocardial ischemia, arrhythmia and cardiac morbidity during anesthesia. Lipid raft/caveola domains encompass β_2-adrenergic receptors, but not β_1-adrenergic receptors for signal transduction (12,51). Mizogami et al prepared SUVs with POPC, SM, POPE, CB and cholesterol (1:1:1:1:2 molar ratio) to compare the membrane effects between different β-blockers at 0.2 and 1 mM by measuring FP (52). Nonselective propranolol most potently increased the fluidity of raft model membranes, followed by alprenolol and oxprenolol, but not β_1-selective atenolol, metoprolol and esmolol. In a similar FP study using SUVs prepared with 33.3 mol% cholesterol and 66.7 mol% phospholipids consisting of equimolar DOPC, SM, POPE and CB, nonselective propranolol and alprenolol increased the fluidity of raft model membranes at 20-200 μM, whereas β_1-selective landiolol and esmolol were not effective even at 200 μM (53). Nonselective β-blockers could reduce the activity of β_2-adrenergic receptors by fluidizing the membrane lipid rafts together with antagonizing β_1-adrenergic receptors by interacting with β_1-adrenergic receptor proteins, producing nonselective blockade of β-adrenergic receptors. In contrast, selective β_1-blockers do not affect β_2-adrenergic receptors through interaction with lipid rafts, thereby enhancing the selectivity for β_1-adrenergic receptors.

Beta-blockers, particularly β_1-selective agents, have been used for treating hypertension (54). Although the altered vascular signaling processes are implicated in hypertension, whether lipid rafts/caveolae are responsible for such pathogenic events remains unclear (55), so no significant interaction between antihypertensive drugs and lipid rafts was found in the literature.

Alpha-adrenergic agonists

Alpha$_2$-agonists with the sedative, analgesic, anesthetic-sparing and sympatholytic activity are used as an adjuvant for anesthesia. Mizogami and Tsuchiya performed FP experiments to investigate their effects on SUVs that were prepared with 33.3 mol% cholesterol and 66.7 mol% phospholipids (consisting of equimolar DOPC, SM, POPE and CB) to be raft model membranes and with cholesterol and phospholipids of different compositions to be neuro-mimetic and cardiomyocyte-mimetic membranes (56). Dexmedetomidine interacted with the non-raft membranes to increase their fluidity most potently at 5-200 μM, followed by levomedetomidine and clonidine. However, these α_2-agonists exerted much weaker effects on the raft model membranes so that dexmedetomidine and levomedetomidine did not show large difference in membrane interactivity despite being significantly different in sedative activity between medetomidine enantiomers.

TABLE III - Interaction of membranous receptor- and enzyme-acting drugs with lipid raft membrane domains and membranes

Drug class	Drug	Membrane	Induced membrane modification	Reference
Adrenergic receptor-acting drug	Nonselective β-blockers (0.2 and 1 mM) Selective β_1-blockers (0.2 and 1 mM)	SUVs (POPC, SM, POPE, CB and cholesterol, 1:1:1:1:2 molar ratio)	Nonselective propranolol most potently increased the membrane fluidity, followed by alprenolol and oxprenolol, but not β_1-selective atenolol, metoprolol and esmolol	52
Adrenergic receptor-acting drug	Nonselective β-blockers (20-200 μM) Selective β_1-blockers (20-200 μM)	SUVs (33.3 mol% cholesterol and 66.7 mol% phospholipids of equimolar DOPC, SM, POPE and CB)	Nonselective propranolol and alprenolol increased the membrane fluidity, but not β_1-selective landiolol and esmolol	53
Adrenergic receptor-acting drug	Alpha$_2$-agonists (5-200 μM)	SUVs (33.3 mol% cholesterol and 66.7 mol% phospholipids (DOPC, SM, POPE and CB))	Dexmedetomidine increased the fluidity of non-raft membranes most potently, followed by levomedetomidine and clonidine, although the effects on raft model membranes were much weaker without showing large difference between medetomidine enantiomers	56
Opioid receptor-acting drug	Rats injected with morphine (25 mg/kg, i.p.)	Hippocampus and caudate membranes	Increased the membrane fluidity	59
	Rats injected with naloxone (2 mg/kg, i.p.)	Hippocampus and caudate membranes	Decreased the membrane fluidity	
	Morphine (10 nM and 10 μM)	Rat brain membrane preparations	Increased the membrane fluidity	
	Naloxone (1 nM)	Rat brain membrane preparations	Reversed the membrane-fluidizing effects of 10 nM morphine	
Opioid receptor-acting drug	Codeine (0.1 M) ***N***-Methylcodeine (0.1 M)	DPPC MLVs	Reduced the phase transition temperature	60
Opioid receptor-acting drug	Etorphine (10 nM)	Human embryonic kidney cells expressing μ-receptors	Translated μ-receptors from lipid rafts to non-raft regions	61
	Mice injected with etorphine (5 μg/kg, s.c.)	Hippocampi isolated after drug injection		
Cyclooxygenase-acting anti-inflammatory drug	Aspirin (3 mM)	DPPC bilayer membranes containing 32.5 mol% cholesterol	Increased the membrane fluidity Disrupted the membrane organization and prevented raft formation	63
Cyclooxygenase-acting anti-inflammatory drug	Aspirin (10 mol%)	MLVs (70 mol% DMPC and 30 mol% cholesterol)	Bound to raft-like L_o phase domains and disturbed their organization	64
Cyclooxygenase-acting anti-inflammatory drug	Indomethacin (5 μM) Naproxen (25 μM) Aspirin (50 μM) Ibuprofen (150 μM)	Baby hamster kidney cells	Affected the organization of raft-like ordered lipid and protein membrane nanoclusters	65

CB = cerebroside; DMPC = 1,2-dimyristoylphosphatidylcholine; DOPC = 1,2-dioleoylphosphatidylcholine; DPPC = 1,2-dipalmitoylphosphatidylcholine; MLV = multilamellar vesicle; POPC = 1-palmitoyl-2-oleoylphosphatidylcholine; POPE = 1-palmitoyl-2-oleoylphosphatidylethanolamine; SM = sphingomyelin; SUV = small unilamellar vesicle.

The mechanistic relevance of lipid rafts to the enantioselective effects of α_2-agonists is inconclusive as Morris et al reported that α_1-adrenergic receptors, but not α_2-adrenergic receptors, occupy membrane lipid rafts (57).

Opioid analgesics

Morphine and its related drugs act on inhibitory opioid receptors of μ, κ and δ subtypes expressed in nociceptive neuronal circuits. Mu-receptors responsible for the effects of opioid analgesics and antagonists are located within lipid raft/caveola membrane domains (58).

Heron et al performed in vivo experiments to inject rats with opioids intraperitoneally and in vitro experiments to subject membranes prepared from rat brains to the reaction with opioids, followed by FP measurements (59). Morphine increased the fluidity of hippocampus and caudate membranes from rats injected at 25 mg/kg (i.p.) and the fluidity of the membrane preparations at 10 nM and 10 μM**.** In contrast, opioid antagonist naloxone decreased the membrane fluidity of the same brain regions at 2 mg/kg (i.p.) and reversed the in vitro membrane-fluidizing effect of 10 nM morphine at 1 nM. Budai et al evaluated the effects of different opioids on DPPC multilamellar vesicles (MLVs) by DSC and electron paramagnetic resonance (EPR) spectroscopy (60). Codeine and *N*-methylcodeine reduced the phase transition temperature of DPPC membranes at 0.1 M. Zheng et al treated HEK (human embryonic kidney) 293 cells expressing μ-receptors with or subcutaneously injected mice with opioid agonists (61). SDGC cell fractions and hippocampus isolates demonstrated that etorphine of 10 nM and 5 μg/kg (s.c.) translocate μ-receptors from lipid rafts to non-raft regions as well as cholesterol-depleting MBC.

Anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs are considered to exert therapeutic and adverse effects by inhibiting cyclooxygenase (COX)-2 and COX-1, respectively. COX-2 is localized in lipid raft/caveola membrane domains and associated with caveolin-1 (62).

Alsop et al studied the effects of aspirin on different DPPC/cholesterol bilayer membrane systems by Langmuir-Blodgett, DSC and ND experiments (63). Aspirin (3 mM) increased the membrane fluidity of DPPC membranes containing 32.5 mol% cholesterol, disrupted the membrane organization and prevented the formation of L_o phase lipid rafts. In the following neutron scattering experiments and molecular dynamics simulations, they prepared MLVs with 70 mol% 1,2-dimyristoylphosphatidylcholine (DMPC) and 30 mol% cholesterol to study the membrane effect of aspirin (64). Aspirin bound to raft-like L_o phase domains and disrupted their organization at 10 mol%. Zhou et al reported that 5 μM indomethacin, 25 μM naproxen, 50 μM aspirin and 150 μM ibuprofen acted on BHK (baby hamster kidney) cells to affect the organization of raft-like ordered lipid and protein membrane nanoclusters by interacting with plasma membranes (65).

Anticancer drugs

In addition to conventional mechanistic effects, anticancer drugs exhibit apoptosis-inducing activity. Lipid rafts contribute to induction of the apoptosis selective for cancer cells (66). Alkylphospholipids, platinum(II) complex and antibiotics are presumed to act on lipid rafts as a membrane gateway to induce apoptosis (67). Alves et al recently published an excellent review on the biophysics of cancer cells and the relevance of drug and membrane interaction to cancer therapy (68). Results of the literature search on the interaction of anticancer drugs with membrane lipid rafts and membranes are shown in Table IV.

Alkylphospholipids

Ausili et al treated MLVs prepared with POPC, SM and cholesterol (1:1:1 molar ratio) with edelfosine at 10-20 mol% relative to membrane lipids, followed by DSC, XD and NMR analysis (69). Edelfosine altered the raft organization and induced the appearance of a sharp phase transition at 20 mol%, suggesting a fluidity increase in membrane lipid rafts. When incubating with human acute T-cell leukemia (Jurkat T) cells, edelfosine colocalized in lipid rafts at concentrations higher than 20 mol%. 10-(Octyloxy) decyl-2-(trimethylammonium) ethyl phosphate (ODPC) with the cytotoxic activity against cancer cell lines inhibits the proliferation of leukemia cells by inducing apoptosis. Gomide et al prepared giant unilamellar vesicles (GUVs) with DOPC, SM and cholesterol (1:1:1 molar ratio) to examine the effects of perifosine and ODPC on lipid rafts (70). In fluorescence microscopic observations, perifosine and ODPC disrupted membrane raft domains in GUVs so that the domains disappeared in less than 1 min after treatment at 100 μM. Castro et al treated MLVs or unilamellar vesicles (ULVs) prepared with POPC, *N*-palmitoyl-SM and cholesterol (1:1:1 molar ratio) with anticancer alkylphospholipids, followed by FA measurements (71). Edelfosine and miltefosine were demonstrated to increase the fluidity of raft model membranes at 5-10 mol% relative to membrane lipids. Wnętrzak et al studied the effects of synthetic phospholipid analog erucylphosphocholine on raft-mimic Langmuir monolayers composed of SM and cholesterol (2:1 molar ratio) (72). Erucylphosphocholine increased the membrane raft fluidity at higher than 0.3 mol% relative to membrane lipids and weakened the interaction between cholesterol and SM. In a thermodynamic study using the same Langmuir monolayers, anticancer 2-hydroxyoleic acid increased the membrane fluidity of raft-mimic monolayers at higher than 0.1 mol% relative to membrane lipids (73).

Cisplatin

Cisplatin acts on plasma membranes to trigger the Fas death receptor pathway at a membrane level (74). Lacour et al treated human colon carcinoma (HT29) cells (7×10^5 cells) with cisplatin at 5 μg/mL for 0.25-4 hours (75). The cells were subjected to 12-DSA (12-doxylstearic acid) spin labeling followed by EPR spectroscopic analysis or cell lysis with Triton X-100 followed by SDGC fractionation and immunoblot

TABLE IV - Interaction of anticancer drugs with lipid raft membrane domains and membranes

Drug class	Drug	Membrane	Induced membrane modification	Reference
Alkylphospholipid	Edelfosine (≥20 mol%)	MLVs (POPC, SM and cholesterol, 1:1:1, molar ratio)	Increased the fluidity of lipid rafts	69
		Human acute T-cell leukemia cells	Colocalized in membrane lipid rafts	
Alkylphospholipid	Perifosine (100 μM) ODPC (100 μM)	GUVs (DOPC, SM and cholesterol, 1:1:1 molar ratio)	Disrupted membrane raft domains	70
Alkylphospholipid	Edelfosine (5-10 mol%) Miltefosine (5-10 mol%)	MLVs or ULVs (POPC, ***N***-palmitoyl-SM and cholesterol, 1:1:1 molar ratio)	Increased the fluidity of raft model membranes	71
Alkylphospholipid	Erucylphosphocholine (≥0.3 mol%)	Raft-mimic Langmuir monolayers (SM and cholesterol, 2:1 molar ratio)	Increased the membrane raft fluidity and weakened the interaction between cholesterol and SM	72
Alkylphospholipid	2-Hydroxyoleic acid (≥0.1 mol%)	Raft-mimic Langmuir monolayers (SM and cholesterol, 2:1 molar ratio)	Increased the membrane raft fluidity	73
Platinum(II) complex	Cisplatin (5 μg/mL)	Human colon carcinoma cells	Increased the membrane fluidity, which was inhibited by 10 μg/mL nystatin pretreatment Translocated CD95 into lipid rafts, which was prevented by 10 μg/mL nystatin pretreatment	75
Platinum(II) complex	Cisplatin (25 μM)	Human colon carcinoma cells	Increased the membrane raft fluidity and induced apoptosis, which was inhibited by cholesterol (30 μg/mL) and monosialoganglioside-1 (80 μM)	76
Antibiotic	Azithromycin (132 μM)	SUVs (DOPC, SM and cholesterol, 1:1:1 molar ratio)	Increased the fluidity of raft-like membranes	77
Antibiotic	Daunorubicin (40-75 μM)	LUVs (DMPC, SM and cholesterol, 7:1.5:1.5 molar ratio)	Decreased the fluidity of raft-like membranes	78
Antibiotic	Doxorubicin (40-75 μM)	LUVs (DMPC and SM, 8:2 molar ratio or DMPC, SM and cholesterol, 7:1.5:1.5 molar ratio)	Increased the fluidity of binary membranes, but not ternary membranes	79

DMPC = 1,2-dimyristoylphosphatidylcholine; DOPC = 1,2-dioleoylphosphatidylcholine; GUV = giant unilamellar vesicle; LUV = large unilamellar vesicle; MLV = multilamellar vesicle; ODPC = 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate; POPC = 1-palmitoyl-2-oleoylphosphatidylcholine; SM = sphingomyelin; SUV = small unilamellar vesicle; ULV = unilamellar vesicle.

analysis. Cisplatin increased the fluidity of plasma membranes as soon as 0.25 hours after the treatment, although its membrane effect was inhibited by pretreating with cholesterol sequestering nystatin at 10 μg/mL. The cell exposure to cisplatin for 4 hours induced the translocation of CD95 (cluster of differentiation 95 known as Fas receptor) into lipid rafts, which was prevented by nystatin pretreated at 10 μg/mL. Rebillard et al treated human colon carcinoma (HT29) cells growing in the exponential phase with cisplatin at 25 μM for 1-72 hours (76). They isolated lipid rafts by SDGC and performed EPR spectroscopic analysis after 12-DSA spin labeling. Cisplatin treatment for 1 hour increased membrane raft fluidity and that for 72 hours induced apoptosis. Such effects were inhibited by membrane-stabilizing cholesterol (30 μg/mL) and monosialoganglioside-1 (80 μM).

Anticancer antibiotics

Berquand et al treated SUVs prepared with DOPC, SM and cholesterol (1:1:1 molar ratio) with macrolide antibiotic azithromycin (77). FP analysis revealed that azithromycin increases the fluidity of a hydrophobic region of raft-like membranes at 132 μM. In FA experiments of Alves et al (78), anthracycline antibiotic daunorubicin (40-75 μM) decreased the fluidity of raft-like membranes of LUVs prepared with DMPC, SM and cholesterol (7:1.5:1.5 molar ratio), while this antibiotic was more effective in decreasing the membrane fluidity of LUVs prepared without cholesterol. Alves et al also investigated the effects of doxorubicin on LUVs prepared with DMPC and SM (8:2 molar ratio) or with DMPC, SM and cholesterol (7:1.5:1.5 molar ratio) by measuring FA (79). Doxorubicin increased the fluidity of binary membranes at 40-75 μM, but not raft-like ternary membranes containing cholesterol.

Phytochemicals

A variety of phytochemicals (bioactive components in plants) such as flavonoids exhibit a broad spectrum of pharmacological activity including antioxidant, antitumor,

anti-inflammatory, analgesic, antimicrobial, cardioprotective, anti-allergic and antiplatelet ones. Many of them with the amphiphilic structure share the property to interact with artificial and biological membranes. The membrane interactivity of phytochemicals was recently reviewed by Tsuchiya (80), especially the interaction of flavonoids with lipid rafts by Tarahovsky et al (81) and their induced changes in membrane fluidity by Selvaraj et al (82). Results of the literature search on the interaction of phytochemicals with membrane lipid rafts and membranes are shown in Table V.

TABLE V - Interaction of phytochemicals with lipid raft membrane domains and membranes

Drug class	Drug	Membrane	Induced membrane modification	Reference
Flavonoid	Quercetin (10 μM) EGCG (10 μM) Cyanidin (10 μM)	SUVs (phospholipids (POPC and SM) and cholesterol by varying the composition 55-80 mol% and 20-45 mol%)	Quercetin decreased the membrane fluidity most potently, followed by cyanidin and EGCG	84
Flavonoid	Quercetin (30 μM)	Human colon cancer cells (HT-29, SW-620 and Caco-2)	Enhanced TRAIL efficacy to induce apoptosis by accumulating death receptors in membrane lipid rafts	85
Flavonoid	Quercetin (10 and 100 μM) Luteolin (10 and 100 μM)	Mouse macrophages	Suppressed the accumulation of lipid rafts to inhibit TNF-α production	86
Flavonoid	Quercetin (2-16 μM)	SUVs (DMPC plus 20 or 33 mol% cholesterol)	Increased the fluidity of raft model membranes	87
Flavonoid	EGCG (5-100 μM)	SUVs (5 mol% cholesterol and 95 mol% POPC or DOPC)	Decreased the fluidity of binary membranes	88
Flavonoid	EGCG (5-20 μg/mL)	Human colon carcinoma cells	Reduced the membrane resistance to Triton X-100 by decreasing ordered membrane domains	89
Flavonoid	EGCG (5 μM)	Human prostate cancer cells	Inhibited $DiIC_{16}$ accumulation in lipid ordered domains and disrupted lipid rafts	90
Flavonoid	EGCG (5-20 μM)	Human multiple myeloma cells	Induced lipid raft clustering and apoptotic cell death	91
Flavonoid	Dimeric procyanidin (0.05-1 μg/mL)	Human acute T-cell leukemia cells	Increased the membrane fluidity	92
Flavonoid	Hexameric procyanidin (10 μM)	Human colon cancer cells	Decreased the membrane fluidity, although the membrane interactivity was lost by MBC (2.5 mM) Prevented the lipid raft disruption induced by MBC or deoxycholate	93
Stilbenoid	Resveratrol (10-80 μM)	LUVs (egg phosphatidylcholine, SM and cholesterol, 1:1:1 molar ratio)	Formed the ordered membrane domains and enhanced the membrane resistance to Triton X-100	94
Anthraquinonoid	Emodin (1-5 mol%) Aloin (1-5 mol%)	MLVs composed of DMPC	Reduced the phase transition temperature	95
Anthraquinonoid	Emodin (10-50 μg/mL)	Human umbilical vein endothelial cells	Disrupted lipid rafts	96
Terpenoid	Ginsenosides Rb2, Rc, Rd, Re, Rf, Rg1, Rg2 and Rh2 (50 μM)	HeLa cells	Increased the membrane fluidity Reduced the raft-marker protein concentration in lipid rafts	98
Terpenoid	Saikosaponin A (3-12 μM)	Mouse macrophages	Inhibited LPS-induced cytokine expression and Toll-like receptor localization in lipid rafts, and reduced membrane cholesterol levels	99

DMPC = 1,2-dimyristoylphosphatidylcholine; DOPC = 1,2-dioleoylphosphatidylcholine; EGCG = (–)-epigallocatechin-3-gallate; LPS = lipopolysaccharide; LUV = large unilamellar vesicle; MBC = methyl-β-cyclodextrin; MLV = multilamellar vesicle; POPC = 1-palmitoyl-2-oleoylphosphatidylcholine; SM = sphingomyelin; SUV = small unilamellar vesicle; TNF = tumor necrosis factor; TRAIL = TNF-related apoptosis-inducing ligand.

Flavonoids

Considering the distribution and accumulation in lipid bilayers, representative flavonoid quercetin and (–)-epigallocatechin-3-gallate (EGCG) possibly alter membrane fluidity and order, making or breaking raft-like domains (83). Tsuchiya and Mizogami compared the effects of different flavonoids on SUVs that were prepared with phospholipids (POPC and SM) and cholesterol by varying their compositions 55-80 mol% and 20-45 mol%, respectively (84). FP data indicated that quercetin interacts preferentially with the hydrophobic region of membranes to decrease the fluidity at 10 μM most potently, followed by cyanidin and EGCG. Psahoulia et al investigated the mechanism underlying an apoptosis-enhancing effect of quercetin by treating human colon cancer cells (HT-29, SW-620 and Caco-2) with quercetin at 30 μM (85). While tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) contributes to apoptosis induction, quercetin enhanced the TRAIL efficacy to induce apoptosis by accumulating death receptors in membrane lipid rafts. In a cell culture study of Kaneko et al, quercetin and luteolin suppressed the accumulation of lipid rafts at 10 and 100 μM to inhibit TNF-α production in mouse macrophages (86). They also suggested that these flavonoids change membrane fluidity. Ionescu et al prepared SUVs with DMPC plus 20 or 33 mol% cholesterol to form the L_o phase and examine the membrane effect of quercetin (87). FP measurements showed that quercetin increases the fluidity of raft model membranes at 2-16 μM.

Tsuchiya treated SUVs consisting of 5 mol% cholesterol and 95 mol% POPC or DOPC with several catechins, followed by FP measurements (88). Of the tested catechins, EGCG most potently interacted with binary membranes to decrease their fluidity at 5-100 μM. Adachi et al stained human colon carcinoma (HT29) cells with fluorescent $DiIC_{16}$ (1,1'-dihexadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) that is preferentially incorporated into the ordered membranes, and then treated the cells with EGCG at 5-20 μg/mL to analyze its membrane effects by fluorescent confocal microscopy (89). EGCG reduced the membrane resistance to Triton X-100 at as little as 5 μg/mL, possibly by decreasing the content of ordered membrane domains. Duhon et al exposed human prostate cancer (DU145) cells to $DiIC_{16}$ in the presence or absence of 5 μM EGCG, followed by fluorescence microscopic analysis (90). EGCG inhibited the accumulation of $DiIC_{16}$ in lipid-ordered domains and disrupted lipid rafts. Tsukamoto et al treated human multiple myeloma (U266) cells with EGCG at 5-20 μM for 3 hours and at 10 μM for 1-3 hours (91). Fluorescence resonance energy transfer and fluorescence microscopic assays indicated that EGCG dose- and time-dependently induces lipid raft clustering and apoptotic cell death.

Procyanidins contained in fruits and vegetables are oligomeric flavonoids with the anticancer activity. Verstraeten et al treated human acute T-cell leukemia (Jurkat T) cells (6×10^4 cells) with cocoa procyanidins and measured FP (92). Dimeric procyanidin increased the fluidity of plasma membranes in a concentration-dependent manner at 0.05-1 μg/mL. In the following experiment, they incubated human colon cancer (Caco-2) cells with 10 μM hexameric procyanidin in the absence or presence of 2.5 mM MBC (93). In contrast to dimeric procyanidin, hexameric procyanidin decreased the fluidity of plasma membranes, although its membrane interactivity was lost by cholesterol-depleting MBC. This procyanidin also prevented lipid raft disruption induced by MBC or deoxycholate (cholesterol depletion/redistribution).

Stilbenoids

Resveratrol present in grape skins and seeds has anticancer, antioxidant and cardioprotective property. Neves et al treated LUVs prepared with egg phosphatidylcholine, SM and cholesterol (1:1:1 molar ratio) with 10-80 μM resveratrol to investigate the effects on raft model membranes by three different methods (94). Resveratrol induced the phase separation and formed the ordered membrane domains at concentrations higher than 10 μM. Such effects were more pronounced in the presence of cholesterol and SM. Resveratrol was also effective at 80 μM in enhancing the membrane resistance to Triton X-100.

Anthraquinonoids

Pharmacological effects of aloe are attributed to anthraquinonoid component emodin and aloin (barbaloin). DSC experiments of Alves et al demonstrated that emodin interacts with MLVs composed of DMPC to reduce the phase transition temperature at 1-5 mol% more potently than aloin (95). Meng et al investigated the mechanism underlying a vascular anti-inflammatory effect of aloe by treating human umbilical vein endothelial cells grown to approximately 90% confluence with emodin (96). Emodin (10-50 μg/mL) inhibited the expression of proinflammatory cytokines and chemokines induced by 0.1 μg/mL lipopolysaccharide (LPS). Similar to cholesterol-depleting MBC (5-12.5 mM), emodin (10-50 μg/mL) disrupted lipid rafts that are relevant to the cell activation by LPS. Lipid raft disruption associated with integrin signaling pathway is also responsible for the inhibitory effects of emodin on tumor cell adhesion and spreading (97).

Terpenoids

Triterpenoid glycosides from *Panax ginseng* and triterpenoid saponin derivatives from *Radix bupleuri* have anti-inflammatory and anticancer activity. Yi et al treated HeLa cells with different ginsenosides at 50 μM and stained the cells with carboxy Laurdan, followed by fluorescence microscopy and generalized polarization imaging (98). Ginsenosides Rb2, Rc, Rd, Re, Rf, Rg1, Rg2 and Rh2 increased the fluidity of plasma membranes as well as cholesterol-depleting MBC (10 mM). When fractionating the HeLa cells by SDGC, ginsenoside Rh2 and MBC reduced the concentration of raft-marker proteins in the raft fraction, indicating that they disrupt lipid rafts. These effects of ginsenoside Rh2 were reversed by cholesterol overloading (20 μg/mL). In a cell culture study of Wei et al (99), 3-12 μM saikosaponin A inhibited the expression of cytokines in primary mouse macrophages stimulated by 0.1 μg/mL LPS. Such inhibitory effects were attenuated by replenishment of 84 μg/mL cholesterol, while 3-12 μM saikosaponin A reduced cholesterol levels in macrophage membranes. Saikosaponin A (3-12 μM) and MBC (10 mM) also inhibited the LPS-induced

localization in lipid rafts of Toll-like receptors that play a crucial role in the innate immune system.

Conclusions

Results of the literature search indicate that different classes of drugs interact with raft model membranes and cellular membrane lipid rafts in addition to interacting directly with membrane receptors, ion channels and enzymes. They could physicochemically modify membrane lipid rafts to be a platform for functional proteins and the membranes surrounding such raft domains, affecting the organizational integrity of lipid rafts with the subsequent alteration of receptor, channel and enzyme activity, thereby producing pharmacological effects. With respect to the induced membrane modification, raft-acting drugs are characterized as ones to decrease membrane fluidity, induce L_o phase or order plasma membranes, leading to lipid raft formation; and ones to increase membrane fluidity, induce L_d phase or reduce phase transition temperature, leading to lipid raft disruption. Targeting lipid raft membrane domains would open a new way for drug design and development.

Given the critical role of lipid rafts/caveolae in cellular signal transduction, odontology may be the promising field to which a raft-targeting concept is applied. Anticancer drugs interact with membrane lipid rafts to affect their physicochemical property and organizational integrity in association with apoptosis induction. Lipid raft membrane domains are responsible for cancer cell adhesion and migration, and the levels of cholesterol-rich lipid rafts are elevated in cancer cells compared with normal counterparts (100,101). While phytochemicals interact with membrane lipid rafts and regulate raft formation (83,85), such interactivity is responsible for their diverse bioactivities including apoptosis induction. Among raft-targeting compounds, alkylphospholipids and flavonoids could be a novel type of anticancer drug.

Since an outbreak of atypical pneumonia was first reported in Wuhan (China) in December 2019, novel coronavirus or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections have spread worldwide, causing a global pandemic of coronavirus disease 2019 (COVID-19). SARS-CoV-2 spike proteins have a strong binding affinity to human angiotensin-converting enzyme 2 (ACE2) (102). Host cell ACE2 receptors, which are a cell-specific target of and responsible for the cellular entry of SARS-CoV-2, are localized in lipid rafts (103). Cholesterol-rich membrane domains are essential for the spike proteins to interact with ACE2 receptors efficiently (104) and cellular cholesterol levels are closely associated with COVID-19 lethality (105). Agents that specifically disrupt ACE2-localizing lipid rafts and deplete raft cholesterol may be a drug to reduce SARS-CoV-2 infectivity and COVID-19 severity.

Abbreviations

L_o, liquid-ordered; L_d, liquid-disordered; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; SM, sphingomyelin; SDGC, sucrose density gradient centrifugation; MBC, methyl-β-cyclodextrin; FP, fluorescence polarization; FA, fluorescence anisotropy; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; ND, neutron diffraction; XD, X-ray diffraction; $GABA_A$, γ-aminobutyric acid type A; NMDA, *N*-methyl-D-aspartate; POPE, 1-palmitoyl-2-oleoylphosphatidylethanolamine; POPS, 1-palmitoyl-2-oleoylphosphatidylserine; GPMV, giant plasma membrane vesicle; LUV, large unilamellar vesicle; DPPC, 1,2-dipalmitoylphosphatidylcholine; DLPC, 1,2-dilauroylphosphatidylcholine; MAC, minimum alveolar concentration; DOPC, 1,2-dioleoylphosphatidylcholine; Nav, voltage-gated sodium; SUV, small unilamellar vesicle; CB, cerebroside; POPI, 1-palmitoyl-2-oleoylphosphatidylinositol; MLV, multilamellar vesicle; EPR, electron paramagnetic resonance; COX, cyclooxygenase; DMPC, 1,2-dimyristoylphosphatidylcholine; ODPC, 10-(octyloxy) decyl-2-(trimethylammonium) ethyl phosphate; GUV, giant unilamellar vesicle; ULV, unilamellar vesicle; EGCG, (–)-epigallocatechin-3-gallate; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; LPS, lipopolysaccharide; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ACE2, angiotensin-converting enzyme 2.

Author contributions

HT designed and conducted the present study and prepared the first draft of the manuscript. HT and MM did literature search, information analysis and manuscript preparation. Both authors reviewed and approved the final manuscript.

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Conflict of interest: Authors disclose no potential conflicts of interest.

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