

Membrane-lipid therapy: a new approach in molecular medicine

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Although most drugs bind to proteins and regulate their activity, some drugs act through a new therapeutic approach called membrane-lipid therapy and bind to lipids, thus modulating the structure of membranes. Most cellular functions are highly dependent on the lipid environment because they are controlled by proteins in or around membranes. The wide variety of cell and organelle membranes and the existence of special lipid regions (e.g. microvilli) and domains (e.g. lipid rafts) support the possibility of designing specific lipid therapies. Indeed, recent evidence suggests that lipid therapy might have potential for the treatment of cancer, cardiovascular pathologies, neurodegenerative processes, obesity, metabolic disorders, inflammation, and infectious and autoimmune diseases.

Introduction

Cellular functions are usually associated with the activity of proteins and nucleic acids. Consequently, human pathologies are classically viewed as malfunctions of these macromolecules. Indeed, most marketed drugs interact directly with proteins and it is increasingly common that new molecular entities for clinical use are developed after elucidating the structure of target proteins. However, recent studies have shown that the types and relative abundance of lipids in the membrane control numerous functions and thus regulate the localization and activity of membrane proteins [1–3]. Because most cellular functions occur in or around cell membranes and because alterations in the types and/or levels of membrane lipids have been described in many human pathologies, it is conceivable that specific therapies could be designed on the basis of regulating membrane lipid structure. The aim of membrane-lipid therapy is to develop drugs that are capable of influencing lipid organization through principles of structure–function, inducing a concomitant modulation of membrane-protein localization and activity. This type of regulation can finally induce changes in cell signaling and gene expression, which might serve to reverse the pathological state. This reversal might even be achieved in the absence of previous membrane-lipid alterations and modulate the membrane structure to control molecular events that are relevant to the pathological state or its therapy.

Thousands of cellular proteins interact with membranes in different ways. Integral (transmembrane) proteins are embedded in the lipid bilayer and their activity is sensitive to changes in the lipid environment [1]. Peripheral (amphitropic, extrinsic) proteins bind to membranes in a reversible manner and their activity is regulated by membrane-lipid organization [2]. Membranes define cells and organelles, and they participate in cellular processes (e.g. biochemical, metabolic, signaling, genetic, detoxifying, sorting, biosynthetic, lytic and recycling processes) and separate the sites where these processes take place. Indeed, the wide diversity of cell types in the human body is reflected in the huge variety of membrane compositions and structures [3]. It is also important to bear in mind that within a single cell membrane, certain proteins associate with locally defined (and sometimes transient) membrane domains, such as coated pits, lipid rafts or synaptosomes. The assortment of lipid types and membrane structures generates an appropriate scenario in which membrane-lipid therapy can be employed. To apply such an approach and to design specific membrane-lipid therapies, several areas are the focus of current research: (i) lipid structure–function; (ii)

Glossary

Integral membrane protein: also known as intrinsic membrane protein. Integral membrane proteins are irreversibly associated with the membrane through transmembrane-spanning regions.

Lamellar phase: this is the most common lipid structure. It is also known as lipid bilayer and defines most phospholipid membranes found.

Lipid polymorphism: lipids can organize into various phases under different conditions (e.g. temperature, pH and ionic strength). This property of lipids is also called lipid mesomorphism. Under certain conditions, several phases can occur simultaneously.

Membrane fluidity: this property of membranes is associated with lipid mobility, which is important for the correct distribution and function of some proteins. Viscosity is an antonymous term often used to refer to the same physical property.

Membrane-lipid therapy: this therapeutic approach is based on the regulation of the membrane-lipid composition and structure to modulate cell functions.

Molecular-shape model: this model explains the tridimensional structure of lipids in a simple manner and the membrane structure as a consequence of the 'shape' of lipid molecules.

Nonlamellar phase: this term refers to lipid secondary structures that are different from the lipid bilayer. They include hexagonal, cubic and rhombohedral phases.

Peripheral membrane proteins: also known as extrinsic membrane proteins or amphitropic proteins. They reversibly bind to membranes through lipid co/post-translational modifications or specific amino acid regions that provide the appropriate context for electrostatic and/or hydrophobic interactions. They are relevant in the context of signal transduction and cellular physiology because they can propagate messages from the plasma membrane to intracellular membranous or aqueous compartments.

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protein–lipid interactions; and (iii) the involvement of lipid structure–function and protein–lipid interactions in cellular signaling and pathophysiology. This novel therapeutic approach is not only applicable to rare or infrequent diseases. Indeed, recent discoveries indicate that it could potentially be used alone or in combination with other therapies to treat some modern major pathologies.

The drugs that can so far be considered to act through membrane-lipid therapy usually bind to membrane lipids (Figure 1) [4]. This contrasts with conventional chemotherapy that is based on the interaction of drugs with proteins, although both of these therapeutic strategies share the aim of regulating protein activity. However, during the past few years new therapeutic approaches have been developed whose molecular bases differ from the above and whose use to treat certain disorders seems promising [e.g. gene, small-interference RNA (siRNA), protein-based and stem-cell-based therapy] (Figure 1). Here, we shall discuss membrane-lipid therapy in the context of recent advances in basic and clinical sciences.

Membrane lipid structure

Protein function is controlled by structural principles and, as discussed later, similar structure–function relationships modulate membrane-lipid organization. Although the study of protein structure began in the 1930s [5], the study of membrane lipid structure began only ~30 years ago [6]. The more recent identification of membrane structure underlies the delays in developing therapies that target lipids. The less extensive knowledge of membrane structure and the later application of this knowledge, compared with protein structure, are due to the higher complexity of lipid organization. Whereas proteins are formed by 20 different amino acids and nucleic acids are formed by four different bases, membranes contain thousands of different lipids. Indeed, there is a huge variety of lipid classes [e.g. phospholipids, sphingolipids, lysophospholipids, isoprenoids, glycolipids, free fatty acids (FFAs), ceramides, triglycerides, cholesterol and cholesterol esters], which can in turn be divided into subclasses, each of which comprises a large number of different lipid types. For example, phospholipids include phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol. Furthermore, phosphatidylethanolamine can have the same or different fatty acids (FAs) at the stereo-specific numbering sn1 and sn2 positions. Thus, if we consider only the 20 most abundant FAs, the number of different phosphatidylethanolamine (or other phospholipids) types reaches 400, although their relative abundance is variable.

Membrane lipids have different physical properties (sometimes even within the same lipid class) in terms of, for example, fluidity, cross-sectional area, electric charge, molecular weight and nonlamellar-phase propensity. Similar to proteins and nucleic acids, the properties of membranes result from the contribution of smaller components. However, lipids are not covalently bound in membranes but rather they interact dynamically to form transient arrangements whose stability can vary. This phenomenon constitutes another source of complexity for their structural study. Furthermore, membrane lipids are

polymorphic: that is, they can exist in a variety of different organized supramolecular structures. The number of possible secondary structures formed by lipids is greater than the number formed by proteins and nucleic acids (Figure 2a,b).

Although the lamellar phase (see Glossary) (lipid bilayer) is recognized as the most common lipid arrangement in cells, to date several types of lamellar structures have been described. The fluid lamellar phase (also called L_α or liquid crystalline) is the structure found in most membrane regions and domains (Figure 2c); in L_α structures, the hydrocarbon chains that form the membrane core exhibit a high level of mobility. Under different conditions, lipids can organize into more-ordered bilayers, such as the L_β (gel) structure, L_c (pseudo-crystalline) bilayers or P_β (rippled) membranes (Figure 2c) [3]. Lipid rafts segregate from the rest of the membrane, forming defined domains within the so-called liquid-ordered (l_o) phase [7] (Figure 2d). In the l_o phase, the acyl chains of lipids are extended and tightly packed as in the gel phase, but they have a higher degree of lateral mobility. By contrast, L_α (frustrated) membranes have different physical properties because they contain high levels of nonlamellar-prone lipids that apply little lateral pressure on the polar (headgroup) regions of the membrane. This feature enables some phospholipids to adopt a splayed (extended) conformation, whereby one of the phospholipid acyl chains extends out from the bilayer, whereas the other remains within the membrane [8] (Figure 3b). Therefore, in the L_α and l_o organizations different proteins are associated with the membrane: L_α domains define regions of interaction between G-protein-coupled receptors (GPCRs) and heterotrimeric $G\alpha\beta\gamma$ proteins, whereas l_o regions form domains where the $G\alpha$ subunits of G proteins interact with and regulate ‘effector’ proteins (e.g. adenylyl cyclase) [2].

In addition to the different types of bilayers described, membrane interdigitation and nonlamellar phases add versatility to lipid structures (Figure 2). In healthy cells, nonlamellar structures are rare, although the presence of these structures or the propensity of membranes to adopt them is associated with important cellular functions. Lipid-curvature stress controls the organization of lipids into various types of hexagonal phases (H_I and H_{II}), cubic phases (micellar and bicontinuous) and rhombohedral phases, among others. (Figures 2 and 3) [3]. The ‘molecular shape’ model [9] helps explain the role of membrane lipids in membrane structure. In this model, phosphatidylcholine resembles a cylinder (Figure 2a), indicating that the cross-sectional area of the polar head is similar to that of the acyl chains. The apposition of cylinders gives rise to a monolayer and, in an aqueous environment, lipid monolayers tend to organize into the well-known bilayer structure to stabilize the hydrophobic regions of phospholipids. Other lipids have different cross-sectional areas for their hydrophilic and hydrophobic regions. For example, diacylglycerol and phosphatidylethanolamine have small polar heads and, thus, their molecular shapes resemble truncated cones (Figure 2a). When aggregated, these lipids form nonlamellar inverted monolayers (hexagonal phase H_{II}), rhombohedral phases or complex tridimensional

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curved bilayers (bicontinuous cubic phases) (Figure 2b). By contrast, lipids that have a greater cross-sectional area in the hydrophilic region tend to form other structures (e.g. cubic micellar or hexagonal H_I). The presence of such nonlamellar-prone lipids in biological membranes regulates the properties of the bilayer. Thus, phosphatidylethanolamine, which is abundant in the cytosolic leaflet of the membrane, increases the nonlamellar-phase propensity (negative curvature strain) that is necessary for some cell-signaling processes [2]. Although nonlamellar-prone lipids usually form bilayers, they can transiently form nonlamellar structures under certain conditions.

One important feature of biological membranes is the existence of cross-sectional and lateral asymmetries. On the one hand, the cytosolic monolayer contains high levels of phosphatidylserine and phosphatidylethanolamine, whereas the extracellular monolayer is rich in phosphatidylcholine and sphingomyelin (cross-sectional

asymmetry). On the other hand, several types of cells are highly polarized (lateral asymmetry). Thus, epithelial and endothelial cells, in addition to certain glandular cells (e.g. acinar cells), have well-defined basal, lateral and apical membranes with specific lipid and protein compositions. When the 'different' membrane region is small compared with the surrounding areas (like an island in the sea), this is referred to as a membrane domain. Several types of membrane domains, such as lipid rafts, caveolae, coated pits, receptor and channel clusters and synaptosomes have been described. Some of these domains are stable (e.g. synaptosomes), whereas others are transient or mobile (e.g. lipid rafts) [10,11] (Figure 2d). Thus, it is clear that lipids and the structures they form are diverse and provide special features to membranes that are necessary to maintain numerous functions.

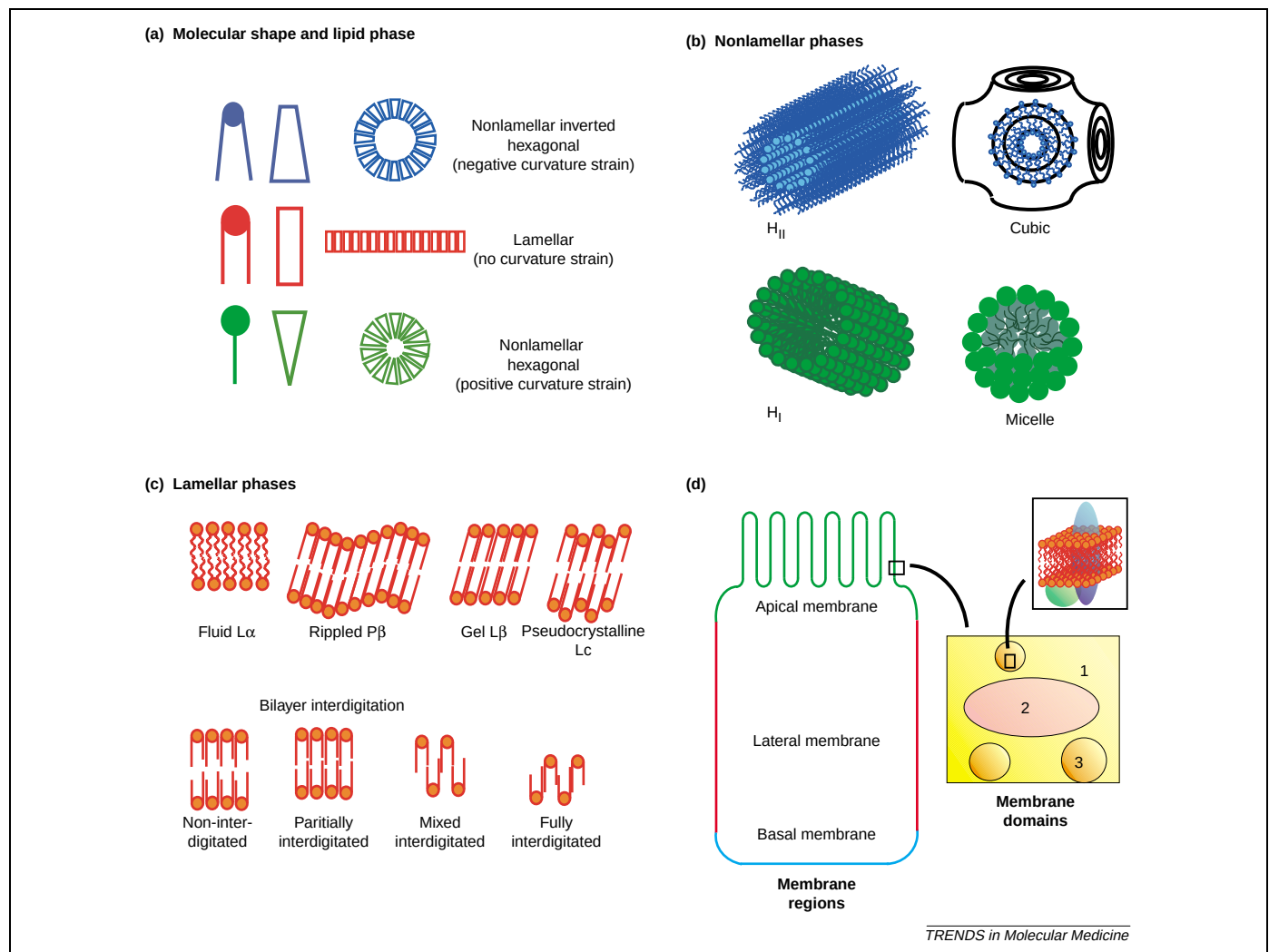
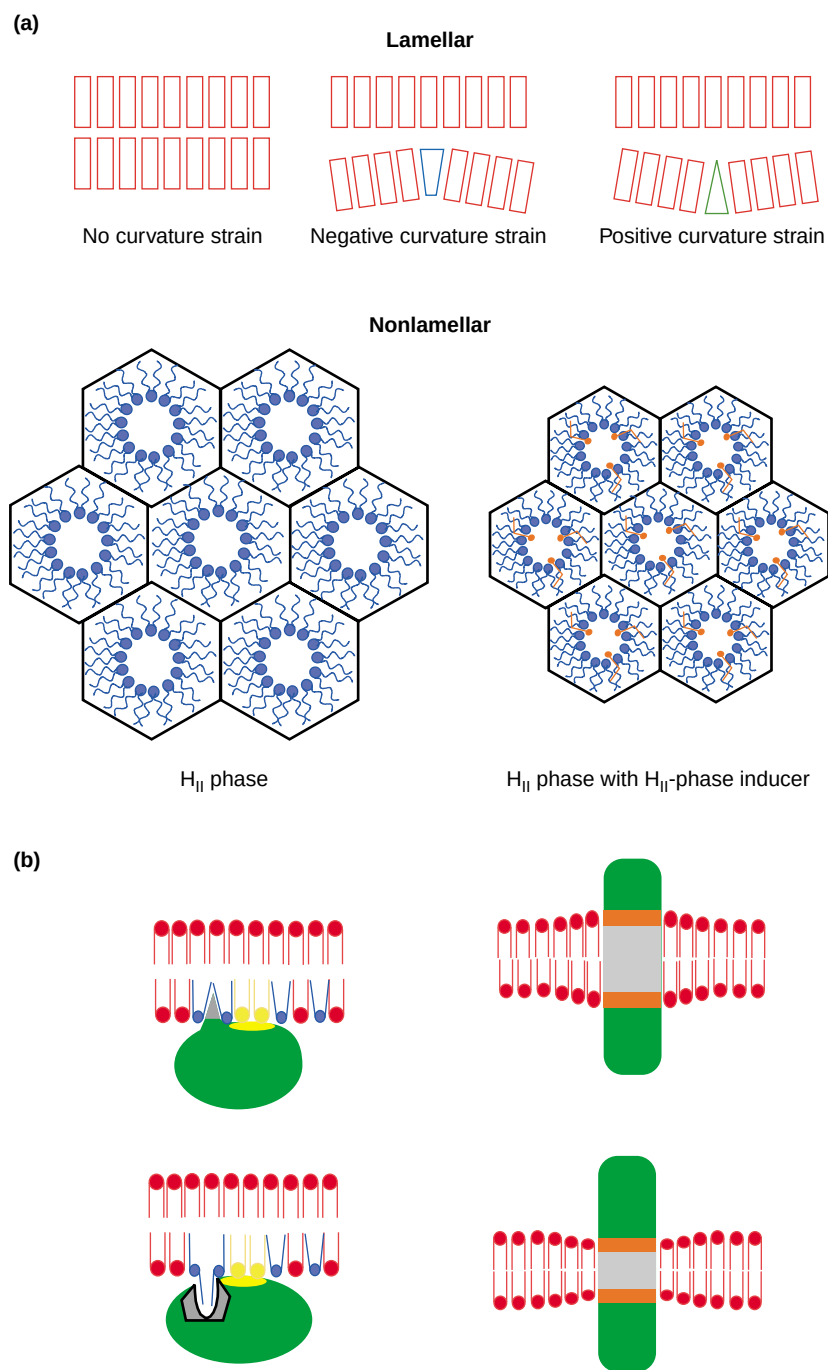


Figure 2. Membrane lipid structure. Examples of lipid shapes and their influence on membrane structure. (a) Lipids with a small polar head (blue), such as phosphatidylethanolamine, have a molecular shape that resembles a truncated cone. They induce a negative curvature strain and favor the organization of membranes into inverted micelles (H_{II} phases) or cubic (bicontinuous) structures (b). Lipids with a bulky polar head and only one acyl chain (e.g. lysophospholipids) (green) have a molecular shape that is similar to an inverted cone and induce a positive curvature strain in membranes. They favor the formation of tubular (H_I) or spheric micelles (b). Lipids such as phosphatidylcholine have similar cross-sectional areas for the polar head and hydrophobic region and resemble cylinders (red). They form lamellar phases (lipid bilayers), without curvature strain. Lipid bilayers can form several different structures, some of which are depicted in (c). (d) In many polarized cells (e.g. small-intestine endothelial cells) there are specialized [e.g. apical (green), lateral (red) and basal (blue)] regions that contain specific lipids and proteins. In a given membrane area (1), GPCR-cluster domains (2) and lipid-raft domains (3) can be well differentiated.



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Figure 3. Membrane lipid structure modulation and protein–lipid interactions. (a) Hexagonal (H_{II})-prone molecules (blue) induce a negative curvature strain on a lipid bilayer (red), whereas H_I-prone lipids (green) induce a positive curvature strain. The cross sections of H_{II} phases are shown below in blue. Hexagonal (H) phases are so-called because they form structures that tend to group with a hexagonal lattice. A molecule [e.g. minerval (red)] that increases the curvature strain in a H_{II} phase induces a reduction of the micelle diameter (right). (b) Modes by which peripheral (left) or integral (right) proteins might interact with membranes. The small polar head of H_{II}-prone lipids (blue) might enable: (i) the hydrophobic regions of the amphitropic protein to penetrate into the apolar regions of the bilayer (upper panel); and/or (ii) the protein to dock to the bilayer via acyl chains from phospholipids in the extended conformation (lower panel). The data available [2] suggest that the latter mechanism is used by heterotrimeric G proteins for binding to membranes. In addition, polar regions of peripheral proteins (yellow) interact electrostatically with charged membrane phospholipids, such as phosphatidylserine (yellow). Differently, the hydrophobic core (grey) and polar regions (red) of the transmembrane sections of integral proteins in part determine the thickness of the bilayer and influence the membrane curvature strain. In addition, these peptide regions might have special lipid requirements and also change the structural properties of membranes.

Lipid structure and cell function

If the only purpose of membranes was the generation of a molecular barrier and a support for proteins, then phosphatidylcholine and cholesterol would be enough to produce a selective and consistent lipid bilayer. However,

this passive view of the membrane has been superseded by recent discoveries showing that membrane lipids are involved in crucial cell functions that justify the presence of many different lipids. Their tight regulation and adaptation to environmental changes argue in favor of

their relevance in the physiology of the cell. Membrane fluidity was one of the first aspects of lipid structure shown to influence the activity of important proteins [12]. Another property of lipids, the nonlamellar-phase propensity, has since been shown to be involved in recruiting heterotrimeric G proteins to the vicinity of the receptor for signal amplification (L ϵ -like membranes) (Figure 3) [2,13]. By contrast, some G α monomers that are generated by G-protein dissociation prefer l $_o$ -like phases, as in lipid rafts where they interact with effector proteins [2,14]. G $\beta\gamma$ dimers retain their affinity for nonlamellar membrane regions close to GPCRs [2]. Upon dissociation from G proteins, they bind GPCR kinases (GRKs), which phosphorylate and inactivate receptors [15]. Therefore, nonlamellar-phase propensity is involved first in the recruitment of a large number of G-protein molecules and then in sorting the G α and G $\beta\gamma$ protein subunits.

In addition, membrane lipids are not the only molecules to influence membrane structure. Membrane-interacting proteins can also modulate the lipid secondary structure, thus regulating the local properties of lipid bilayers (Figure 3b). For example, the transmembrane regions of membrane receptors and the isoprenyl moieties of G-protein γ -subunits increase the nonlamellar-phase propensity of membranes [16,17]. Pre-active heterotrimeric G proteins prefer nonlamellar-prone membranes, so that receptor-rich membrane regions (with a high density of transmembrane peptides) favor the presence of G proteins whose isoprenyl moieties induce the binding of additional transducer molecules. Thus, nonlamellar-phase propensity helps to generate the correct context for signal transduction and signal amplification upon receptor activation by neurotransmitters or hormones. In addition, these and other co/post-translational lipid modifications of membrane proteins (i.e. myristoylation and palmitoylation) appear to be relevant in their interactions with membranes [18]. In this context, certain protein–lipid interactions have been known for several years. For instance, some protein kinase C (PKC) isoforms are activated by phosphatidylserine, phosphatidylethanolamine and diacylglycerol (DAG) through specific interactions and by structurally influencing membrane-lipid organization [4,19–21]. Phospholipase C is a membrane protein activated by G proteins that cleaves phosphatidylinositol into inositol phosphate and DAG. DAG, similar to phosphatidylethanolamine, promotes PKC translocation to membranes, which is usually associated with enzyme activation, whereas phosphatidylinositol and phosphoinositides are important in the context of cell signaling through other pathways (e.g. Ca²⁺) [22].

Nonlamellar-phase propensity also regulates the activity of several relevant cell-signaling proteins, such as $\alpha_{2A/D}$ -adrenoceptors, G proteins, adenylyl cyclase and PKC. [23–25]. Furthermore, nonlamellar structures are involved in membrane fusion and fission, processes that occur frequently in cells [26]. Lamellar- and nonlamellar-prone lipids can form distinct membrane regions and domains that establish the appropriate environment for specific types of protein activity and at the edges of which membrane defects that favor some processes arise

[10,11,20]. Phosphatidylethanolamine and phosphatidylserine are found mainly in the inner leaflet of the membrane. Phosphatidylethanolamine is responsible in part for the nonlamellar-phase propensity of the regions where it accumulates. Among other functions, negatively charged phosphatidylserine provides the appropriate environment for electrostatic interactions between proteins and membranes. The cross-sectional distribution of these lipids is important for cells; indeed, the loss of asymmetry is associated with apoptosis, blood coagulation and a wide variety of health disorders [27]. Finally, certain membrane-lipid domains also act as temperature sensors involved in cellular responses to heat shock [28]. All these studies show the relevant roles of membrane lipids and lipid structures in cellular functions.

Modulation of membrane lipid structure

The participation of membrane lipids in cellular activities indicates that they might constitute targets for drugs whose pharmacological effects would be associated with the modulation of the physicochemical properties of membranes. How can this be achieved? Intercalation of lipophilic or amphiphilic compounds into the membrane can regulate membrane structure [29] (Figure 3). However, these interactions have usually been considered to follow bulk-phase thermodynamics. Recently, numerous studies have shown that structure–function relationships similar to those that control the activity of proteins are applicable to membrane lipids [11,23]. Eighteen-carbon FAs provide an interesting example of the structural bases of lipid regulation. Whereas the *cis*-unsaturated FA oleic acid ('boomerang-shaped') increases the H_{II} nonlamellar-phase propensity (negative monolayer curvature) (Figure 3a), its *trans*-unsaturated analog elaidic acid ('rod-shaped') does not significantly alter the membrane structure. Interestingly, the behavior of the latter is closer to that of stearic acid (a saturated 'rod-shaped' FA). Thus, differences in the 'molecular shape' of these FAs are more important than chemical differences (e.g. the presence or absence of a double bond and the corresponding loss of hydrogen atoms) in regulating membrane structure [30,31]. Accordingly, oleic acid, but not elaidic and stearic acids, alters the activity of signaling membrane proteins [23]. In addition to lipids, other membrane-binding molecules, such as α -helical peptides, might have a role in regulating membrane structure [32].

One question that remains to be fully addressed is the potential specificity of membrane-lipid therapy compared with conventional therapies that target proteins. The necessary degree of specificity might be generated through the great diversity of lipid compositions, structures and arrangements in biological membranes, providing an appropriate scenario for highly specific lipid therapies. Furthermore, it must be borne in mind that many target proteins for clinical drugs are widely distributed in cells and, therefore, drugs act on both healthy and unhealthy cells. Thus, it is possible that these two therapeutic approaches share similar specificities.

Membrane-lipid therapy in the treatment of human pathologies

The use of lipids or the modulation of their levels in serum to treat certain metabolic disorders is known as lipid therapy [33]. Although this therapeutic strategy can regulate the composition and structure of membranes, it is conceptually different from membrane-lipid therapy. Membrane-lipid therapy is a novel therapeutic approach aimed at developing drugs to regulate membrane-lipid composition and/or structure. Some insight already exists into the effects of dietary lipids [34] or certain drugs (e.g. anesthetics and alcohols) [35] on membrane structure and function, but lipids had not previously been considered as targets for drug development. Enzymes and GPCRs constitute the two most common targets for marketed and developing drugs [36]. Many enzymes and GPCRs, and related proteins, interact with membranes and might be modulated by specific lipid therapies. Because the type and/or composition of membrane lipids is altered in several pathologies (Table 1) and an important number of cellular functions occurs within or around membranes, membrane-lipid therapy might have potential use for the treatment of several illnesses.

Alterations in membrane-lipid composition and structure appear to be related to the development of various

cardiovascular pathologies, such as hypertension, atherosclerosis, coronary heart disease, sudden cardiac death, blood vessel integrity and thrombosis [37–40]. In this context, oleic acid increases the H_{II} propensity of membranes and regulates the activity of GPCRs, G proteins and effectors that control blood pressure [23,30,31] and are altered in hypertensive subjects [37]. The lamellar and nonlamellar phases regulate protein–lipid interactions of G-protein subunits [2]. Moreover, oleic acid modulates G-protein function through its activity on lipid structure because it does not modify the activity of pure G proteins in the absence of membranes [23]. This effect, a prototype of the membrane-lipid therapy, is highly structurally specific because elaidic and stearic acids (oleic acid congeners) do not induce similar effects. Accordingly, a higher olive oil consumption (~80% of oleic acid) is associated with a lower risk of developing cardiovascular pathologies [34]. Recently, a synthetic monounsaturated FA that is structurally analogous to oleic acid (2-hydroxyoleic acid) was shown to exert a potent hypotensive effect [25].

Cancer is characterized by several widely diverse alterations in signaling, the nature of which depends on the type of cancer. In this context, a wide variety of molecular entities involved in the control of cell

Table 1. Human pathologies and lipid abnormalities^a

Disease	Membrane abnormality ^b	Proposed molecular mechanisms	Refs
Cardiovascular (hypertension)	Changes in membrane phospholipid and cholesterol levels; changes in FA levels	Regulation of the membrane structure with concomitant alteration of membrane signaling, protein localization and activity	[37]
Cardiovascular (sudden cardiac death)	Changes in membrane levels of saturated and unsaturated FAs	Alterations in δ -6-desaturase activity in the coronary artery wall	[38]
Cardiovascular (cardiac hypertrophy)	Changes in membrane levels of triacylglycerol types and other lipids	Changes in cell signaling and impaired triacylglycerol availability	[39]
Cancer (pathological proliferation)	Changes in membrane FA levels	Altered cell structure and function (including cell proliferation)	[41]
Cancer (multidrug resistance)	Alterations in phospholipid species (PS) levels	Reduced drug intake and facilitated drug removal from cancer cells	[43]
Respiratory pathologies	Changes in membrane microdomains lipid composition	Alterations in mechanotransduction and other signaling processes	[70,71]
Renal pathologies	Increased lipid peroxidation and augmented proportions of saturated FAs caused by hemodialysis	Increased cellular oxidative stress	[72]
Alzheimer's disease, aging and neurodegeneration	Reduced levels of PUFAs in brain cell membranes	Altered expression of transthyretin and other genes related to learning, cognitive and integrative functions	[64,65]
Inflammation, autoimmune and related diseases	Release of pro-inflammatory lipids from membranes	Formation of eicosanoids from arachidonic acid; changes in membrane fluidity; changes in membrane lipid–protein interactions	[69]
Infectious diseases	Increased ceramide-enriched membrane domains	Modified membrane-lipid domains act as platforms for a wide variety of viruses, bacteria and parasite infections	[68]
Schizophrenia	Decreased proportion of PUFAs in membrane phospholipids	Myelin-related and neurotransmitter signaling dysfunctions	[74]
Obesity	Changes in membrane lipids	Alterations in membrane protein function	[53]
Alcohol-induced fetal damage	Changes in cell membrane composition	Various cell functions alterations	[73]
Coagulation (Scott syndrome)	Defective PS flip-flop translocation in membranes	Impaired interaction of coagulation factors and blood cell membranes	[27]
Triose phosphate isomerase deficiency	Lack of symptoms is associated with modification of membrane lipids and lipid fluidity	Changes in membrane protein–lipid interactions and in the activity of certain enzymes	[75]

^aSome representative lipid alterations in most major pathologies and some rare health disorders are shown.

^bAbbreviations: PS, phosphatidylserine; PUFAs, polyunsaturated fatty acids.

proliferation and survival are membrane-associated proteins. Indeed, the levels of membrane lipids are altered in cell membranes from patients with cancer [41,42] and from cancer cells that are resistant to cancer chemotherapy [43]. Therefore, it is not surprising that the activity of various anticancer drugs is associated with their ability to alter the membrane lipid structure. Indeed, daunorubicin, hexamethylene bisacetamide and minerval all alter the non-lamellar-phase propensity of membranes [4,19,44]. Edelfosine [Et-18-OCH₃ (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine)] and miltefosine [HePC (hexadecylphosphocholine)] are also targeted to membranes [45]. The development of new membrane-targeted anticancer drugs is based on the knowledge that: (i) anthracyclines exert their cytotoxic activity solely through interaction with the plasma membrane [46]; and (ii) anthracyclines modify PKC- and GPCR-associated signaling by regulating membrane structure [19]. Similarly, the antitumoral drugs hexamethylene bisacetamide and minerval (oleic-acid derivative) also regulate membrane-phase structure and PKC activity [4,44,47]. Interestingly, a higher oleic acid (i.e. olive oil) intake has been associated with a reduced risk of cancer in humans [48]. Lipid analogs of conventional antitumoral drugs have recently been developed. One example is NEO6002, a less toxic citarabine derivative with a cardiolipin moiety that binds to membranes and overcomes resistance to citarabine in cancer cells [49]. In fact, the use of lipids as targets to overcome anticancer drug resistance has been highlighted recently [42]. Moreover, novel lipid derivatives of drugs that were developed initially for other purposes are currently being investigated for their potential use as antitumoral agents (e.g. FA derivatives of the anesthetic propofol) [50].

Obesity, a disorder that is reaching epidemic proportions in industrial countries, is associated with an increase in the incidence of cardiovascular pathologies, cancer and diabetes, in addition to a general rise in mortality rates [51]. Obesity does not simply involve an accumulation of triacylglycerols and an expansion of the number of adipocytes because numerous reports have also shown alterations in the membrane-lipid composition of different cell types in obese subjects [52]. These lipid modifications induce changes in the activity of membrane proteins, owing to variations in the physical properties of lipids [53]. In this context, adrenoceptors are involved in the control of body weight [54,55] and are differentially regulated by *cis*- and *trans*-unsaturated FAs present in membranes [23]. It is interesting that intake of *cis*-unsaturated FAs is associated with loss of body weight, whereas intake of saturated or *trans*-unsaturated FAs has the opposite effect [56]. Several reports have shown that a high intake of a given FA is reflected in an increase of membrane-lipid types that contain the FA, so that the FA can influence the activity of signaling systems. Therefore, lipids can regulate appetite and satiety through mechanisms that involve membrane signaling, and they can be used in the treatment of obesity, diabetes and related metabolic diseases [57]. In addition to these mechanisms, FAs regulate body weight and metabolism

through binding to peroxisome proliferator-activated receptors (PPARs) [58].

Following adipose tissue, neural tissue has the second highest content in lipids. Hydrophobic drugs readily cross the blood–brain barrier, supporting the potential use of membrane-lipid therapy to treat health disorders in the CNS. ApoE lipoproteins (abundant in the brain) influence the lipid composition of membrane rafts. ApoE abnormalities in Alzheimer's disease patients result in changes in cell signaling owing to alterations in the cell-membrane-lipid composition [59]. These changes in membrane lipids result in alterations of the physicochemical properties of membranes and of signaling protein activity that are relevant to the etiology of this pathology [60,61]. The β -amyloid precursor is a membrane protein and the resulting proteolytic fragments, associated with development of Alzheimer's disease, form deposits on the extracellular side of the membrane. Changes in the lipid composition of membranes have been shown to regulate lipid–protein interactions and the toxicity of β -amyloid peptides [62]. Indeed, it is noteworthy that some treatments and diets supplemented with phospholipids and FAs have beneficial effects on the mental status of patients with Alzheimer's disease and animal models of aging [63,64]. Moreover, lipid interventions are reflected in the composition of brain membrane lipids, which in turn regulate first cell signaling and then gene expression, inducing improvements during neurodegenerative processes [64,65]. In this context, phosphatidylserine alters the interaction of the Tau protein with neuron membranes, which changes the proportion of Alzheimer's disease-like epitopes with respect to other epitopes [66] and regulates the formation of amyloid fibers [67]. In addition, membrane-bound cholesterol reduces the toxicity of β -amyloid protein peptides [62]. These studies suggest that neurodegeneration is influenced by membrane lipid-related protein misfolding, offering further support for the use of membrane-lipid therapy in the treatment of Alzheimer's disease and other neurodegenerative disorders.

Membrane lipids have also been involved in infectious diseases. Indeed, lipid rafts enriched in ceramide are used as platforms by several microorganisms to infect human cells [68]. In this line, NK-2 is a hydrophobic membrane-binding peptide antibiotic that regulates the structure of the membrane and kills bacterial but not human cells [32]. This is due to the different composition and/or structure of eukaryotic and prokaryotic cells and indicates that specific lipid therapies might be applied to combat infectious diseases and to overcome resistance to conventional antibiotics. Furthermore, animal experiments and clinical studies indicate that omega-3 FAs might have anti-inflammatory properties and therefore might be useful in managing inflammation, asthma and other autoimmune diseases [69]. In addition, membrane lipids have been implicated in mechanotransduction and related respiratory signaling processes, and lipid-binding molecules can be used in the treatment of respiratory pathologies [70,71]. Therefore, membrane-lipid therapy gives rise to new possibilities for the treatment of diseases

that currently still require therapies or alternative or improved therapeutic solutions.

Concluding remarks

Membrane-lipid therapy is a new therapeutic approach in which the molecular targets are the lipids and the structures they form. This pharmacological strategy aims to regulate cell functions that are controlled by membrane-associated proteins. The variety of biological lipids, the different structures they form and the alterations of membrane lipids in numerous pathologies suggest that specific lipid therapies could be developed to treat human pathologies. Here, I have shown that membrane-lipid therapy might have potential for the treatment of cancer, hypertension, obesity, neurodegenerative pathologies, metabolic disorders, infectious diseases, inflammation, autoimmune diseases and possibly other health disorders. Future studies will determine the full potential of this new therapeutic approach.

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