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Editorial

Membrane-lipid therapy: A historical perspective of membrane-targeted therapies — From lipid bilayer structure to the pathophysiological regulation of cells☆

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ABSTRACT

Our current understanding of membrane lipid composition, structure and functions has led to the investigation of their role in cell signaling, both in healthy and pathological cells. As a consequence, therapies based on the regulation of membrane lipid composition and structure have been recently developed. This novel field, known as Membrane Lipid Therapy, is growing and evolving rapidly, providing treatments that are now in use or that are being studied for their application to oncological disorders, Alzheimer's disease, spinal cord injury, stroke, diabetes, obesity, and neuropathic pain. This field has arisen from relevant discoveries on the behavior of membranes in recent decades, and it paves the way to adopt new approaches in modern pharmacology and nutrition. This innovative area will promote further investigation into membranes and the development of new therapies with molecules that target the cell membrane. Due to the prominent roles of membranes in the cells' physiology and the paucity of therapeutic approaches based on the regulation of the lipids they contain, it is expected that membrane lipid therapy will provide new treatments for numerous pathologies. The first on-purpose rationally designed molecule in this field, minerval, is currently being tested in clinical trials and it is expected to enter the market around 2020. However, it seems feasible that during the next few decades other membrane regulators will also be marketed for the treatment of human pathologies. This article is part of a Special Issue entitled: Membrane Lipid Therapy: Drugs Targeting Biomembranes edited by Pablo V. Escribá

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1. Introduction

The molecule 2-hydroxyoleic acid was designed on the basis of the divergent effects of oleic acid (OA) and its analogues on membrane structure, and its similarities with the biophysical effects of daunorubicin (an anti-cancer drug formerly called daunomycin) on the structure and function of membrane lipids, and on signaling proteins [1–3]. At that point it would have been difficult to explain the tremendous eclosion of Membrane lipid therapy (MLT), a new field centered on the development of molecules to regulate membrane lipids in order to treat not only cancer but also neurological pathologies, metabolic disorders, cardiovascular conditions, etc. [4]. To the best of my knowledge, 2-hydroxyoleic acid was the first MLT compound rationally designed to treat a condition by modulating membrane lipid composition and structure. This design was based on two previous discoveries. On the one hand, a new anti-tumor mechanism of action reported for anthracyclines that was based on the regulation of the plasma membrane structure, regulating membrane signaling protein localization and activity [3,5]. On the other hand, the fact that OA appeared to be as strong as anthracyclines in regulating the membrane lipid structure and activity of membrane signaling proteins [1,6,7].

Abbreviations: 2OHOA, 2-hydroxyoleic acid; AD, Alzheimer's disease; Aβ, amyloid peptide; CHO, cholesterol; CNS, central nervous system; DAG, diacylglycerol; DHA, docosahexaenoic acid; DHA-H, hydroxydocosahexaenoic acid; EA, elaidic acid; EGFR, Epidermal Growth Factor Receptor; EPA, eicosapentaenoic acid; FASN, fatty acid synthase; FA2H, fatty acid 2-hydroxylase; FDA, food and drug administration; FRAP, Fluorescence Recovery After Photobleaching; GMP, Good Manufacturing Practice; GPCR, G protein-coupled receptor; HSP, heat shock protein; HSR, heat shock response; HTO, hydroxytriolein; ICMT, isoprenylcysteine carboxymethyltransferase; LDL, low density lipoprotein; l_d, liquid disordered bilayer; l_o, liquid ordered bilayer; MLT, membrane lipid therapy; MUFA, monounsaturated fatty acid; OA, oleic acid; PD, Parkinson's disease; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; PAF, platelet activating factor; PUFA, polyunsaturated fatty acid; RCE1, Ras converting enzyme 1; ROS, Reactive Oxygen Species; SA, stearic acid; SFA, saturated fatty acid; SM, sphingomyelin; SCI, spinal cord injury; SMS1, SM synthase 1; TRP, transient receptor potential; TRIB3, tribbles-3 pseudokinase; VLDL, very low density lipoprotein.

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Although MLT is a relatively recent term, molecules that regulate membrane lipid structure have been used for a long time in medicine. Thus, the pharmacological actions of substances like chloroform (and probably other general anesthetics) and ethanol, used for therapeutic purposes, are mediated by the regulation of the membrane lipid structure and membrane-related signaling events [8]. In addition, more recently developed drugs, like the hydroxylamine derivative bimoclomol, can also regulate the lipid structure of the membrane, thereby modulating the localization and activity of relevant proteins in connection with their therapeutic effects in diabetes [9]. The role of the membrane's biophysical properties in the control of heat shock protein (HSP) regulation has been further demonstrated by the effect of the bimoclomol analogue, arimoclomol, in the treatment of lysosomal storage diseases. In this context, arimoclomol is effective in treating Niemann-Pick type C disease by regulating the membrane's structure and the ensuing activation of HSP70 [10], leading to its designation as an orphan drug for the treatment of this condition (European Medicines Agency orphan designation EU/3/14/1376; 2014). In addition, other molecules rationally designed to target membrane lipids, such as minerval or 2-hydroxylinoleic, have been studied in clinical trials in humans (ClinicalTrials.gov identifier #NCT02201823) [11], and the synthetic fatty acid 2-Hydroxydocosahexaenoic acid (DHA-H) reached the market in 2015 (DHALifort, PharmaConcept) [12]. This review will highlight relevant milestones in this recent therapeutic field, focusing on the molecular bases underlying the roles of membrane lipids in cells, the factors that define the regulation of membrane lipid structure, the molecular determinants governing the therapeutic effects of MLT drugs, and their regulatory and pharmaceutical status.

2. A historical voyage to the discovery of the membrane and beyond

Our advances in understanding membrane lipid structure and function have been crucial to design and develop new therapies specifically targeting lipid bilayers. In this review, some of the important discoveries that have contributed to our current understanding of the role of membranes in cells will be mentioned. The *Cell Theory* states that the minimum autonomous live system is a cell, all organisms being formed by a number of them [13]. The nature of the biological entity that defines cells, the plasma membrane, has been of great interest to scientists for a long time. The earliest studies on the structure of membranes and water-lipid interfaces can be found in old texts by Pliny the Elder (55 CE), preceding those of Benjamin Franklin (1722), and more recently those of Agnes Pockles (1891) and Irving Langmuir (1917) [14]. Thus, Pliny the Elder described that seafarers used cooking oils, which could spread over a wide area of water to still rough seas. This work was continued by Benjamin Franklin, who added one teaspoon of oil on water or on a table. He observed that while oil could cover a half acre surface on the lake, it did not spread on a polished marble surface, indicating the relevance of water-oil interactions. He could have discovered the thickness of a trioein monolayer (10 Å) but this was reported 120 years later by Lord Rayleigh. Ms. Pockels' experiment, explained in a letter sent to Lord Rayleigh that eventually was published in Nature, described what would be known as a Langmuir trough decades later [14]. In addition, relevant works on the structure and function of cell membranes were published by William Hewson (1773), Wilhelm Pfeffer (1877), Charles Ernest Overton (1899) and Evert Gorter (1925). On the one hand, Hewson discovered that red blood cells undergo osmotic swelling when water is added to the medium, deducing the existence of a plasma membrane as a structure surrounding the liquid protoplasm [15]. On the other hand, Pfeffer's osmotic pressure experiments suggested the existence of a cell structure he called the plasma membrane. Moreover, the membrane theory was proposed by Wilhelm Pfeffer in 1877 [16], although previous works from Hooke and Leeuwenhoek suggested the existence of a membrane surrounding cells [17,18]. Overton defined membranes as semi-permeable structures probably containing cholesterol (CHO) and phospholipids (PLs) that could prevent certain molecules from entering cells while allowing other molecules to cross membranes in a passive or "uphill" manner (active, against gradient) [19]. In addition, his study on the correlation between the efficacy of an anesthetic and membrane permeability can be considered as the first research into MLT, which was also reported independently by H.H. Meyer (Meyer-Overton theory) [20,21].

Gorter defined the membrane as a lipid bilayer after showing that the surface of lipids extracted from erythrocytes covered a Langmuir monolayer with a surface that doubled the membrane area of the erythrocytes used in the experiment [22]. Luckily, he used a simple cell model without complex internal membranes that would have masked his results. In 1935, Danielli and Davson proposed their model of a biological membrane [23]. Their Pauci-Molecular model was the first to describe a lipid bilayer surrounded by globular proteins like a sandwich (Fig. 1) and it was derived from the experiments carried out by Gorter and Grendel [22]. Based on Robertson's electron micrographs, this model later evolved to explain the structure of membrane pores (Unit Membrane model) adding transmembrane spanning protein regions [24]. In this model, all membranes were constituted into a lipid bilayer with proteins covalently bound on both sides to form a tri-laminar structure (railroad tracks). This Danielli-Davson-Robertson model was not compatible with many of the properties of biological membranes but importantly, it did introduce the concept of membrane asymmetry.

In addition to the plasma membrane, other membranes define relevant cell compartments and organelles. Based on their studies in mitochondria, Green and colleagues proposed that a membrane was a two-dimensional continua of nesting, repeated lipoprotein units [25,26]. This protein-crystal model explained the hydrophobic interactions between globular transmembrane proteins and lipids, yet it underestimated the presence and relevance of the lipid bilayer and peripheral proteins in membranes (Fig. 1). A similar lipoprotein model was proposed by Benson based on his studies of thylakoid membranes (Fig. 1) [27].

In the early 1970s, a variety of approaches provided additional information on the structure of membranes. In this context, an iconic paper by Singer and Nicolson proposed the Fluid Mosaic model, which defined the membrane structure as an orientated two-dimensional viscous solution of amphipathic proteins (or lipoproteins) and lipids in thermodynamic equilibrium [28]. With some modifications, this model is still widely accepted today because it accounts for most of the structural and functional properties of membranes [29,30]. According to this model, integral and peripheral membrane proteins are asymmetrically distributed across a dynamic lipid bilayer (Fig. 1).

Another relevant concept that developed in the years that followed the proposal of the fluid mosaic model is the existence of Membrane microdomains. Membrane domains or microdomains could be defined as finite and dynamic patches in membranes that have a specific lipid and protein compositions distinct to the membrane areas that surround them. As such, they have distinct biophysical features from their surrounding areas (Fig. 2) and some studies in the 1970s had already highlighted the lateral heterogeneity of membranes due to the immiscibility of specific lipid phases [31,32]. These studies helped establish that lipids do not yield homogeneous mixtures in membranes, an observation that at that time was striking given that most lipids are in a liquid (crystalline) state under physiological conditions, suggesting they would diffuse freely in membranes.

Membrane domains had been clearly defined by 1982 (Fig. 2) [33], although the discovery of detergent resistant membrane microdomains, called lipid rafts, arose much interest (Fig. 2) [34]. Several type of liquid ordered (l_o) membrane domains have been identified, and they differ in terms of the type of lipids and proteins that accompany sphingomyelin and CHO, typical components of these lipid raft microdomains (Fig. 2B). Although most microdomain studies have focused on lipid rafts, other microdomains with less organized lipids (liquid disordered domains, l_d) can also be observed using different experimental approaches (Fig. 2). Each of these microdomains has a typical subset of proteins so that they can participate in propagation of different cell signals.

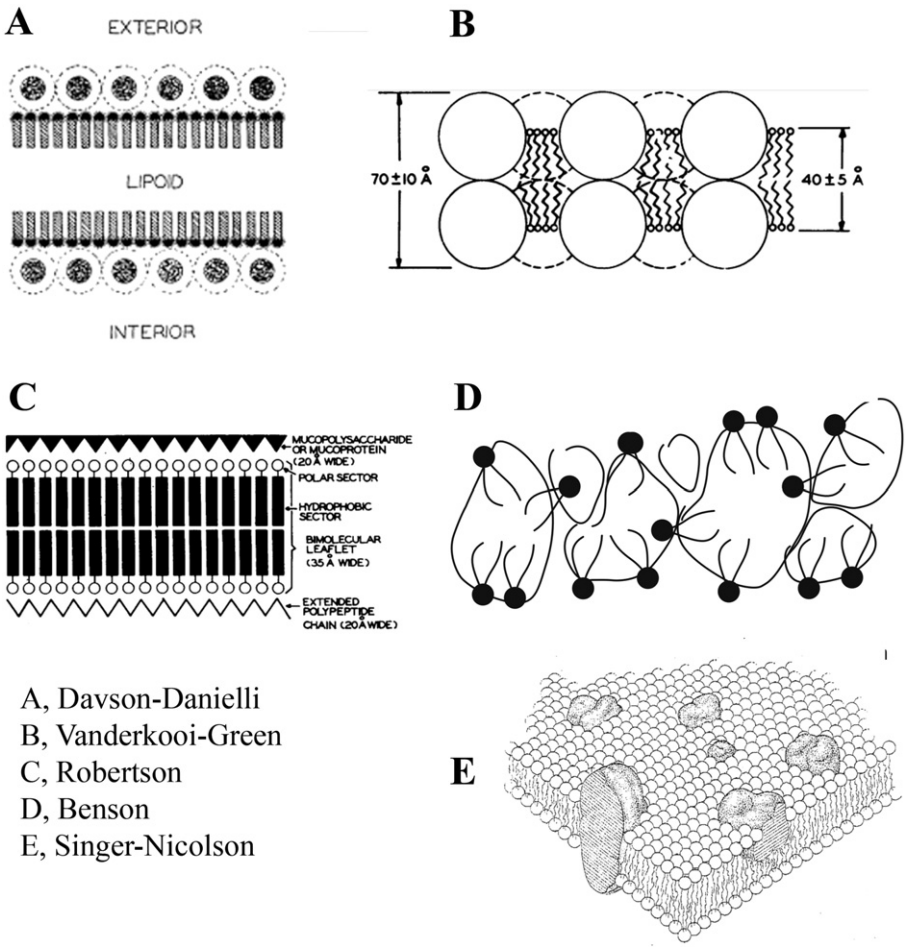

Fig. 1. Evolution of the membrane models during the XXth Century. A, the Davson-Danielli model [23]. The Vanderkooi-Green model [26]. C, the Robertson model [180]. D, the Benson model [27]. E, The Singer and Nicolson fluid mosaic membrane model [28].

The biophysical properties of membrane microdomains in general, and of lipid rafts in particular, have been studied through different approaches. Combining biophysical techniques with the development of phase diagrams has been very useful to understand how each lipid contributes to the overall behavior of a given domain, and how different lipid combinations contribute to the structural and functional properties of membranes and the microdomains therein [35,36]. In contrast to highly organized rafts, which are rich in sphingomyelin (SM) and CHO, nonlamellar-prone domains are rich in different lipids, such as phosphatidylethanolamine (PE) or diacylglycerol (DAG) [37]. In these domains, the presence of other lipids like C26-PC confers stability to the highly curved membrane microdomains [38].

In summary, the different lipid species found in membranes influence the membrane's properties, including the formation of microdomains, which are crucial for the cell's physiology. Indeed, alterations in lipid composition can provoke pathological processes, which can in turn be regulated using membrane-targeted therapies (i.e.: MLT). If a lipid bilayer containing a single PC species with one saturated and one unsaturated acyl chain is stable, permeable and flexible, why do we need thousands of lipids in our membranes and why do the proportions of these change in pathophysiological circumstances? Moreover, why are these elements organized into such a wide array of microdomains and membrane regions? Specific interactions of proteins with either discrete lipid species or organized lipid structures in part account for the need for so many lipids [39]. Furthermore, each cell organelle has a characteristic membrane composition, and each cell type may have different lipids in their plasma and other membranes. In addition, not only the overall composition but also the transbilayer lipid observed in cell membranes can be influenced by the activity of numerous enzymes [40–42]. In this context, alteration of the extracellular layer of the membrane with lectin induces important changes in the organization of the intracellular layer, which further supports the fluid nature of membranes and stresses the impact that transbilayer structural alterations may have on the functions of membranes [43].

3. Involvement of the membrane lipid fraction in specific cell functions

The plasma membrane has been seen by most scientists as a physical barrier in which lipids perform a mainly structural function and proteins undertake the relevant (catalytic) tasks. Although this view of the lipid bilayer prevailed until recent years, early studies carried out in the 1970s clearly indicated that membrane fluidity allowed lipids and proteins to move relatively freely along membranes, which could regulate their activities. Using immunofluorescent antibodies, it was shown that proteins could mix by means of lateral diffusion in heterokaryons formed by the fusion of human and mouse cells (obtained using sendai virus), which depended on the temperature [44]. Experiments using fluorescence recovery after photobleaching (FRAP) further demonstrated the extent of membrane fluidity [45].

There is no question that the biophysical properties of membrane lipids are responsible for the control of relevant membrane functions and the activity of pivotal proteins that regulate a cell's physiology. Subtle changes in the structure of lipids and their abundance in membranes have important consequences for the structure and function of cell membranes. For example, we can consider the effect of three common fatty

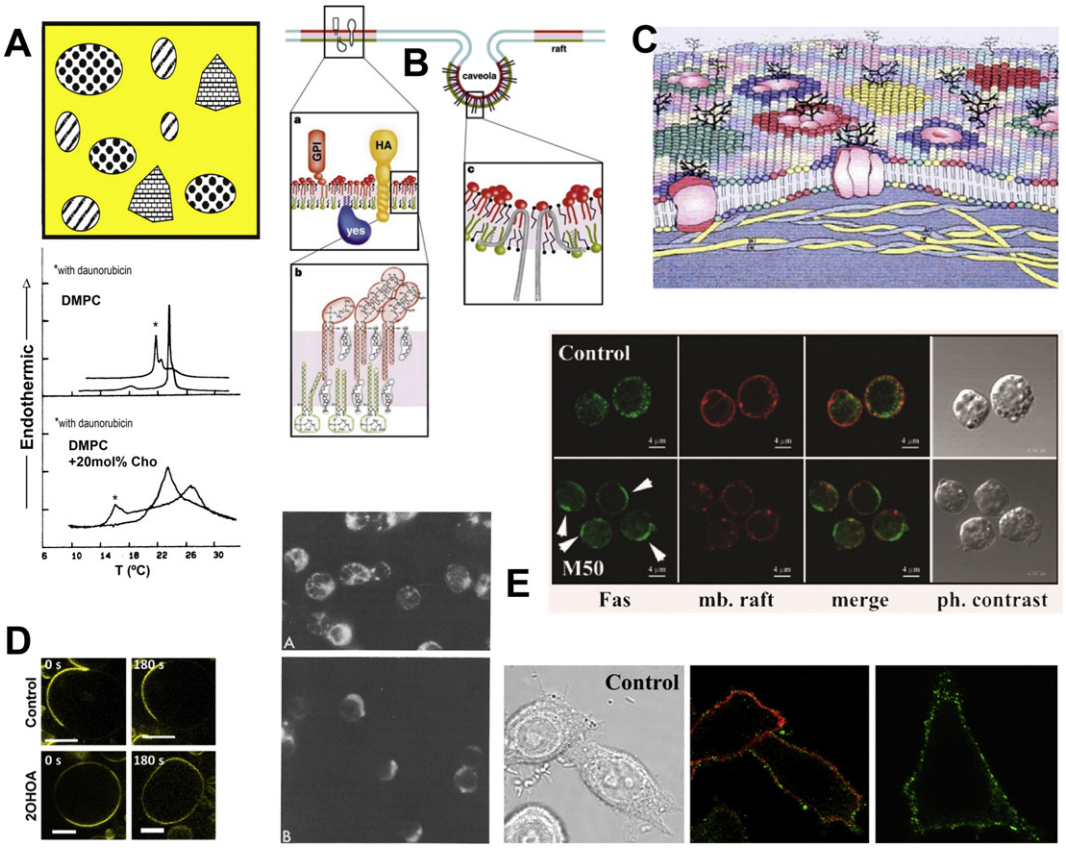

Fig. 2. Current view of the membrane. A, Differential Scanning calorimetry showing that different lipids and therapeutic agents can form membrane microdomains, and their representation (adapted from P·V. Escribá Ph.D. Thesis, 1988). Deconvolution analysis of the calorimetric peaks demonstrated the heterogeneity of model dimirystoyl phosphatidylcholine (DMPC) membranes without (upper graph) or with CHO (lower graph) and in the absence or presence of the anticancer drug daunorubicin (*) B, Lipid rafts and other types of membrane microdomains in the membranes contain specific lipids and proteins with particular structures and functions [34]. Microdomains are dynamic in their protein and lipid composition and functions. C, Multiple types of microdomains with lipids, proteins and sugars form in membranes, and they interact with the cytoskeleton [181]. These microdomains are depicted by different colors that indicate the different composition of contiguous membrane microdomains. D, MLT drugs, such as 2OHOA (in this figure) or 2-hydroxylinoleic acid can alter the domains formed in giant unilamellar vesicles [182]. Thus, DiI (yellow) mainly labels l_d domains. 2OHOA caused an important reduction in the size of the huge lipid raft observed in control membranes and also a reduction in the total raft abundance (from ca. 50% to ca. 40%) in POPC:PE:SM:CHO (1:1:1:1, mol ratio). E, Natural and synthetic lipids can control the localization of tubulin [33], the Fas Receptor (green; membrane label: red) [183] or Ras protein (green; membrane label: red) to specific membrane microdomains [51]. Both FasR and Ras localizations were regulated by 2OHOA.

acids in membranes: stearic acid (SA), OA and elaidic acid (EA). While SA is a saturated fatty acid (SFA), OA and EA are monounsaturated fatty acids (MUFAs), with double bond in the *cis* or *trans* conformations, respectively. In a compact lipid layer, SA and EA would adopt a straight rod shape, whereas OA would have a kink as a result of its *cis* double bond. This simple fact has an important consequence on the bilayer's structural features as OA is excluded from lipid rafts and it brings greater fluidity to the membrane as well as an increased molecular area and nonlamellar-phase propensity, while reducing the membrane thickness [6,46]. The structural difference between OA and EA (the latter being closer in structure to SA) not only has distinct effects on the bilayer's structure but also, on the activity of membrane proteins, many of these interacting preferentially with OA- or EA-rich microdomains. Thus, the activity of α_{2A}-adrenergic receptors and G proteins is strongly regulated by the presence of OA in membranes but not by SA or EA [1]. In addition, the regulation of membrane structure and function by lipids containing polyunsaturated fatty acids (PUFAs) differs from that produced by MUFAs [46,47]. These results indicate that in addition to their role as singular entities, the role of lipids in supramolecular structures (e.g., membrane microdomains) is also relevant in membranes.

Each major membrane lipid, such as CHO, SM, PE, phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), in their various proportions and forms, provide membranes with specific biophysical properties that collaborate to define their structure and function. These lipids, and the lipid structures they form, participate in interactions with proteins' hydrophobic moieties and residues (lipid-lipid and lipid-protein interactions, respectively) in membranes. These interactions give rise to a variety of membrane microdomains with a defined composition and responsible for activities that regulate specific cell functions, producing lateral and cross-sectional membrane asymmetry [39,42,48]. Thus, negatively charged phospholipids (e.g., PS, PI, phosphatidic acid [PA]) participate in the interactions of peripheral proteins with cationic amino acid-rich regions [49], and lamellar- and nonlamellar-prone phospholipids recruit proteins with either SFA or isoprenyl moieties, as described formerly [50,51] (Fig. 2E) and also in the present special issue (see below). Moreover, the presence of the myristoyl moiety facilitates the interaction of $G\alpha i_1$ with membrane domains rich in PS, a phospholipid with a net negative charge, whereas the presence of the reversible palmitoyl moiety induces rotation of the C-terminal alpha helix that exposes uncharged amino acids to the membrane surface, facilitating the exit of $G\alpha i_1$ protein from microdomains with net negative charge [49]. Therefore, the membrane lipid composition in general and the relative abundance of PS, PE, CHO, etc., influences the type of proteins that associate with the plasma membrane, thereby regulating specific molecular processes that define more general cell functions.

In this context, it is interesting to note that brain membranes from fishes that live in rivers have a different lipid composition during the summer and winter, when the temperature can shift from 4–5 °C to

23–25 °C. This has been associated with the need of membranes to have specific biophysical properties to keep the cells functioning [52]. Specifically, it has been seen that PE containing the MUFAs OA (18:1), arachidonic acid (20:4) and docosahexaenoic acid (DHA, 22:6), are critical to retain the membranes functional properties. These examples of the relevance of membrane lipids in cell functions shed light on how modulating the lipid fraction may serve to devise new therapies. In fact, each lipid alteration observed in cells can be used as a potential target for drug development. Either direct actions to modify lipid structure or indirect effects on enzymes that synthesize or control cell lipids could serve as new therapies following MLT principles [53].

4. How can membrane-lipid targeted therapies be efficacious and specific?

Until recent years, pharmaceutical companies were not interested in molecules targeting membrane lipids simply because, in a world dominated by drugs targeting proteins and nucleic acids, their experts could not understand the technology behind MLT. It was assumed that molecules that change the lipid composition or structure of membranes would be unspecific. However, not only have MLT drugs been shown to be efficacious but frequently, they are more specific and safe than what are considered to be conventional drugs. Thus, the fatty acid 2-hydroxyoleic acid has proven to be efficacious against cancer without inducing the adverse side-effects of other anticancer drugs, indicating the absence of undesired interactions with off-targets [51,54] (ClinicalTrials.gov identifier #NCT01792310). Up to five different MLT mechanisms have been described by which compounds may regulate the cell membrane composition to produce therapeutic effects [53]. In this context, 2-hydroxyoleic acid uses type 1 and type 2 mechanisms: (1) direct interaction with membranes; and (2) indirect regulation via sphingomyelin synthase 1 (SMS1), an enzyme that controls the membrane lipid composition [53,55]. The net result of treatments with 2-hydroxyoleic acid is a relevant increase in the levels of this synthetic fatty acid in membranes and important changes in the levels of membrane phospholipids, mainly the product of SMS1 (SM) and its substrates (PE and PC) [55,56]. Other MLT mechanisms of action have been described: Gene expression regulation via lipid interactions with transcription factors or DNA (type 3), changes in the abundance, size or composition of membrane microdomains, which alters protein-membrane and protei-protein interactions at the membrane (type 4), and regulation of enzymes that catalyze lipid anchor (e.g., fatty acyl and isoprenyl moieties) addition to membrane proteins (type 5) [53].

The role of membrane lipids in controlling the heat shock response (HSR) is also of interest. Unsaturated fatty acids favor a status that maintains a good HSR, which can be mimicked by molecules like benzyl alcohol and its derivatives, compounds that have therapeutic effects in the treatment of diabetes [57]. The effect of certain bioactive molecules on the membrane would mimic the regulatory effect of hyperthermic therapies on the biophysical properties of the bilayer. Compounds such as bimoclomol and other hydroxylamine derivatives can regulate membrane lipid microdomains, another mode of action described for MLT drugs [53]. Indeed, these compounds are of potential therapeutic interest for the treatment of cardiac ischemina, diabetic wounds, neurological disorders, retinopathy and nephropathy [58,59]. These molecules act as co-inducers of the HSR by modifying the membrane structure. Moreover, they have a cytoprotective effect that can be applied to pathophysiological conditions that benefit from HSR activation and HSP regulation [9].

Another interesting means to obtain therapeutic effects via MLT is to regulate the interaction of signaling proteins with membranes. Impairing transducer binding to membranes would interfere with the propagation of signals from receptors to effectors. This approach has been used to develop therapies against conditions in which G proteins are involved. A number of drugs have been discovered, either in screenings or through rational design, to inhibit enzymes that control the binding of the small G protein, Ras, to membranes. Ras proteins are involved in cell proliferation, propagating messages from tyrosine kinase growth factor receptors to signaling cassettes like Raf-MEK-ERK and PI3K-Akt-mTOR. Both Raf and PI3K are kinases that activate downstream molecular entities (e.g., MEK or Akt, respectively), which in turn also regulate the activity of other molecular entities involved in cell proliferation. These regulatory processes require the physical interaction of these proteins at the membrane (e.g., a receptor-Ras and Ras—Raf interaction). Inhibition of the enzymes that modify the CAAX C-terminal motif that is necessary for Ras and other G proteins to interact with the membrane (see below) has stimulated clinical studies into farnesyl transferase, geranylgeranyl transferase, isoprenylcysteine carboxymethyltransferase (ICMT) and Ras converting enzyme (RCE1) inhibitors [60]. Similarly, the interaction between receptors (GPCRs) and G proteins can be modulated by peptides that regulate protein-protein interactions, and that can penetrate cells through lipid anchors that also facilitate their membrane localization (e.g., palmitic acid) [61]. These peptides, called pepducins, can regulate the activity of GPCRs (e.g., protease-activated receptors, chemokine receptors, sphingosine 1-phosphate receptor-3, melanocortin-4 receptor, relaxin receptor, etc.) and they are therefore potential therapeutic agents to combat cancer, cardiovascular conditions, metabolic diseases, etc. [62,63].

5. A very short (and partial) history of modern pharmacology and MLT

In 1928 Alexander Fleming discovered penicillin when a Petri dish was accidentally left open and a Staphylococcus culture was contaminated by *Penicillium notatum*, which was able to kill the bacteria around its growth area [64]. Although penicillin was successfully used as early as 1930 [65], in was not until 1939 that Chain and Florey were able to synthesize it and demonstrate that the compound was efficacious in mice [66]. In 1942, a team led by Max Tishler at Merck & Co began to synthesize penicillin in large quantities to produce sufficient to save the life of millions during World War 2 and beyond.

In part because this discovery was not given much attention (Fleming was a poor communicator) and in part due to the low amounts that could be obtained from fungal cultures, penicillin suffered a tortuous path from its discovery to its use in humans. During this time, sulfonamide antibiotics began to be used systematically. In mouse models, Prontosil (Bayer AG) was found to be efficacious against a range of bacterial infections during the 1930s, after the organism metabolized the prodrug [67]. In the late 1930s, a myriad of sulfonamide derivatives were produced by many manufacturers, one of them containing diethylene glycol, an analogue of antifreeze. This molecule caused the poisoning of more than one hundred people and caused the FDA (USA's Food and Drug Administration) to publish the "Food, Drug and Cosmetic Act" (1938), establishing the basis for Good Manufacturing Practice (GMP). After the Kefauver-Harris Amendments to this Act (1962), new more stringent standards were applied to the pharmaceutical industry (current GMP, cGMP: United States Code Title 21 Section 351 (a)(2)(B) (FDCA § 501 (a)(2)(b)). The amendments were precipitated by the health crisis in Europe involving the use of Thalidomide, which produced birth defects in over 8000 children.

The discovery of medicinal drugs in nature prompted the start of a still ongoing era of drug screening in biological models, such as enzymatic assays, cultured cells and mice. In this context, Sandoz started a program in 1957 to seek out antibiotics in fungi, with employees taking samples wherever they went, on work or on vacation. Cyclosporine (also written ciclosporin) was obtained from soil samples taken by Hans Peter Frey on a trip to Norway in 1969. This cyclic peptide, isolated from the fungus Tolypocladium inflatum, was initially purposed as an anti-fungal antibiotic, although its narrow spectrum soon ruled out this commercial use. Because microbial metabolites often possess interesting pharmacological activities, Sandoz initiated a screening program in the early 1970s to search for compounds with immunosuppressant activity that could replace Ovalacin, which had a high toxicity that frequently interfered with its therapeutic use. This led to the discovery of

the potential immunosuppressant action of Cyclosporin, which has since been used widely to prevent graft rejection [68].

The history of Cyclosporins serves as an example of two of the most relevant strategies currently used, drug screening and drug re-profiling (or drug repositioning/repurposing). Currently, drug screening takes advantage of advances in: (i) biological models of disease that can be rapidly analyzed in initial screenings (mostly cell homogenates or cell culture systems); (ii) complex analytical devices with multiple channels for the simultaneous evaluation of several compounds; (iii) compound (chemical or peptide) libraries; and (iv) programmable robots to replace humans in some steps of the discovery process. Thus, the development of **high throughput screening** has permitted thousands of compounds to be evaluated each year by a single laboratory, constituting one of the main sources of novel drugs. In such processes, molecules that modulate the system in the desired direction are identified, constituting a selection of **hits** that can be subjected to **structural refinement** in order to define **lead compounds** for testing in animals. The processes of **Lead optimization** establish a drug candidate that can enter the preclinical and clinical regulatory phases required to achieve marketing approval if its safety and efficacy is demonstrated. This is a time consuming approach and since any given compound library may not contain the best chemical structure for binding to a given protein or catalytic site, it does not always yield an appropriate molecule. A more recent screening strategy is based on finding small molecular structures that interact with different binding regions or catalytic sites in a target protein and it is called **fragment based drug screening**. Two or more such low-affinity fragments can be bound together with linkers to yield a larger molecule (**hit**) with higher affinity and with a greater therapeutic potential or better features [69].

Another approach to drug discovery involves **rational drug design**. This approach takes advantage of the knowledge gained about protein structure-function relationships, and their roles in cell signaling and pathophysiology. Improved understanding of nucleic acids, and of lipid structure and dynamics, has added further potential sources of rationally designed therapies. Thus, based on the structure of a protein, a nucleic acid sequence or a lipid bilayer, it is feasible to rationally design therapies to treat a wide variety of conditions [53,70,71]. There are two main approaches for rational drug design. First, **structure-based drug design** is based on elucidating the structure of the drug target (protein, nucleic acid or lipid) to design a molecule that regulates its activity, and downstream molecular and cellular events. Thus, X-ray diffraction, NMR, differential scanning calorimetry, fluorescence spectroscopy and other approaches (biochemical, biophysical, molecular biology, molecular modeling, etc.) can be used to determine the 3-D structure of the protein and lipids, whereas nucleic acid sequencing and other approaches are used to determine the sequence/structure of the DNA and RNA targets. Elucidating a receptor binding site or enzyme catalytic site are considered to be the first steps to rationally design drug candidates based on the pharmacophore structure. For example, elucidating the structure of HIV and the hepatitis C virus proteases was fundamental to design and develop drugs for the treatment of these viral diseases [72]. The potential affinity of ligands for receptors can be calculated by molecular modeling, using the relationship between the change in free energy (ΔG) and the binding association constant (K_a):

$$\Delta G = -RT \ \ln K_a$$

where R is the gas constant and T is the absolute temperature [73]. Ideally, the higher the ligand-pharmacophore binding affinity, the higher the efficacy of the compound. Usually, various compounds with relatively high binding probability scores are tested in models to select the best hit. Computer-assisted data have been compared with data obtained by different experimental approaches. Thus, thermodynamic approaches such as isothermal titration calorimetry, can be used to define ligand-receptor binding parameters. In addition, radioligand binding techniques based on separation of bound and free ligand molecules (e.g., filtration, centrifugation, dyalisis, etc.) or spectroscopic techniques which differentiate the optical properties of the bound and free ligand (e.g., absorbance and fluorescence spectroscopy) and other techniques have also been used to determine binding affinities.

Ligand-based drug design is a further way to develop rationally designed drugs, an approach that relies on the knowledge of molecules known to bind to the selected therapeutic target. The structural analysis of known molecules can be used to build computer assisted models of the pharmacophore structure, which in turn can be used to design new compounds. Although these strategies are mainly applied to protein targets, similar approaches can be used to rationally design MLT drugs. Similarly, nucleic acid-based therapies rely on DNA or RNA sequences (e.g., asRNA, miRNA, siRNA, lncRNA, genes in vectors, etc.), and the design of analogous molecules (e.g., a new siRNA with one base difference with respect to a previous siRNA that has therapeutic activity) may also be considered a ligand-based rational design.

In this context, the design of the synthetic anti-cancer lipid, minerval (2-hydroxyoleic acid, 2OHOA, NaCHOleate), shared some common points with drugs rationally designed to interact with proteins using both structure- and ligand-based designs [74]. The design of this compound followed the discovery that anthracycline anti-tumor drugs killed cancer cells by simply interacting with its plasma membrane [75]. Later, it was shown that the anthracycline daunorubicin could bind to membrane lipids [76] and alter the membranes lipid structure [3]. Having shown that **membrane lipid structure could regulate the abundance and type of amphitropic signaling proteins bound to the membrane** [3,5,50], **the lipid bilayer itself (or at least specific microdomains) could be defined as a pharmacophore, and therefore, it could be expected that membrane-binding molecules could have therapeutic potential** [4,53]. In the search for molecules able to regulate the lamellar-to-hexagonal (H_{II}) phase transition similarly to anthracyclines, not only was OA found to be an active compound but also that the closely related analogues, EA and SA, were shown to be inert in terms of this modulation of membrane structure at similar concentrations [6]. This phenomenon argued against the unspecific effects of these closely related fatty acids, further demonstrating that structure-function also applies to drug-lipid and lipid-lipid interactions. Moreover, OA was found to be more potent than the α_2-adrenoceptor agonist UK14304 in regulating signal propagation from GPCR to Gi proteins and in ensuing cAMP production via adenylyl cyclase [1]. Epidemiological studies showing a lower incidence of cancer in populations with high OA consumption were consistent with the potential antitumor activity of this fatty acid [77]. These studies re-defined the view of the beneficial (or detrimental) effects of different fats, establishing the plasma membrane as a new drug target and OA as a pharmaceutical hit. A number of OA analogues were designed, synthesized and tested during the lead optimization process, with 2OHOA the most potent molecule in vitro and in vivo [51,55]. Roman mythology says that Jupiter had a headache that ended when the goddess Minerva was born with weapons from the head of this god. Similarly, the first patient with a brain tumor who was treated with 2OHOA suffered from headaches, which disappeared after a few days of treatment as the tumor faded away. In addition to the efficacy, the lack of adverse side-effects demonstrated that this therapy and this strategy are highly specific and potent.

6. Membrane lipid therapy in clinical studies and human therapy

6.1. Oncology

To the best of our knowledge, 2OHOA was the first rationally designed MLT molecule to enter clinical trials. It has shown good pharmaceutical efficacy and safety against cancer in humans, which rules out unspecific effects due to undesired off-target interactions (ClinicalTrials.gov identifier #NCT01792310). After a first-in-man phase I/IIA trial in patients with solid tumors, 43% glioma patients responded to treatment, although this percentage almost doubled (ca. 80%) if patients previously pretreated with avastin were disregarded. This synthetic fatty acid activates SMS1,

producing relevant reductions in cancer cell membrane PE and increases in SM [55]. In fact, 2OHOA normalizes membrane lipid levels by inducing at least 10-fold changes in the PE:SM ratio in cancer cells but not in normal cells. In normal cells, high product (SM) and low substrate (PC and PE) levels prevent any significant changes in membrane lipid levels [51,55], justifying in part the lack of severe adverse effects at therapeutic 2OHOA doses in humans. Thus, the cancer cell plasma membrane composition, with high PE and low SM levels, would have lower abundance of l_o lipid raft domains and higher abundance of l_d membrane microdomains. Indeed, after treatment with 2OHOA, enhanced membrane order is observed, consistent with an increase in the abundance of lipid rafts, which would be in agreement with the dramatic rise in SM measured in treated cells [56]. In addition to PE, PC levels are also modified by 2OHOA-induced SMS1 activation, although its effects on membrane lipid order (l_o/l_d or raft/non-raft balance) are not as marked as those of SM and PE [55,56]. Indeed, SM mass generated by SMS1 activation upon 2OHOHA treatment has been seen to equal PC plus PE mass loss in tumor cell membranes [55].

As a result of the membrane lipid changes in 2OHOA-treated cancer cells, the oncogenic protein K-Ras (and possibly other proteins) translocates from the plasma membrane to the cytoplasm, shutting down the propagation of proliferation signals from receptor tyrosine kinases (e.g., EGFR) to Raf [51,78]. Subsequently, the Cyclin D-CDK4/6, PI3K-Akt and MEK-ERK proliferation signaling cassettes are inactivated, causing cell cycle arrest and the induction of proliferation- and autophagy-mediated cell death [2,51,78–80]. This behavior supports the role of the membrane as a signaling switch able to induce general changes in the cell's physiology. In this case, the PE:SM ratio functions as a proliferation switch used by normal cells to start or stop cell division, and this switch would become de-regulated in cancer cells. It is possible that other cell switches involving general changes in membrane lipids and that are associated with marked changes in the cell's behavior will be found in the future. The relevance of de-regulating the **lipid proliferation switch** in the development of cancer can be seen in the 10-year survival of glioma patients. The survival of those with weak SMS1 expression is ca. 2%, whereas that of patients with intermediate SMS1 expression is over 20% [54]. Moreover, the median survival of glioma patients with 3 or more mutations in conventional oncogenes and tumor suppressor genes is 22.6 months, whereas 3 or more mutations in membrane SM-related lipid genes causes a marked reduction in the median survival to 14.6 months [54]. Indeed, it was recently reported that inhibition of acid sphingomyelinase, which also reduces PS, would cause similar effects to SMS1 activation on the membrane levels of SM, inducing the membrane-to-cytoplasm translocation of K-Ras [81].

In addition to 2OHOA, 2-hydroxylinoleic acid has successfully finished phase I clinical trial in patients with cancer and it will soon start phase II studies. This PUFA binds to membranes, reducing the size of l_o microdomains and its proportion in vitro (MLT type 4 mechanism: Fig. 4 and reference therein). This lipid also binds to and activates PPARα and PPARγ, which induces the upregulation of Tribbles-3 pseudokinase (TRIB3: MLT type 3 mechanism of action). TRIB3 binding to Akt prevents its activation by upstream kinases, inhibiting the Akt/mTORC1 axis, and it induces specific cancer cell autophagy [11]. Moreover, other PUFAs are undergoing clinical trials to demonstrate their efficacy against cancer. Thus, the safety and efficacy of eicosapentaenoic acid (EPA) and DHA combined (fish oil, 2.5 to 3.6 g daily) is being investigated to prevent colorectal cancer and for its efficacy in patients with gastrointestinal cancer (ClinicalTrials.gov identifiers #NCT01661764 and #NCT02699047, respectively). In addition, DHA covalently bound to the anesthetic propofol and its derivatives has been shown to be efficacious against breast cancer cells [82]. Other lipids, like the alkylphosphocholine phospholipid, miltefosine, are also being tested to demonstrate efficacy against cancer in clinical trials (ClinicalTrials.gov identifier #NCT02366884).

Another interesting synthetic lipid is hydroxytriolein (HTO), an analogue of triolein, which is the most abundant triacylglycerol in olive oil. This triacylglycerol mimetic causes a ca. 2-fold increase of membrane DAG levels and a concomitant reduction of the lamellar-to-hexagonal (H_{II}) phase transition. This effect causes a sustained 2-fold increase of protein kinase Cα translocation to the plasma membrane and its ensuing activation, which produces inhibition of cancer cell growth through the β-catenin pathway [83]. In addition, HTO downregulates the MEK-ERK axis, and it induces the production of Reactive Oxygen Species (ROS) and apoptosis in non-small cell lung cancer cells [83]. Finally, other lipids (e.g., free fatty acids, triacylglycerols, etc.) are also regulated by HTO treatments. Thus, over and above synthetic fatty acid analogues, other types of lipids may have interesting therapeutic effects (e.g., triacylglycerol analogues).

In addition to lipids as possible therapeutic tools to combat cancer, enzymes that regulate lipid metabolism may be targets for anti-cancer therapies. For example, FA Synthase (FASN) is an oncogenic marker in breast cancer patients with poor prognosis, suggesting that it could serve as a therapeutic target against cancer [84]. In fact, the FASN inhibitor, orlistat (Roche Xenical®), blocks growth and induces breast cancer cell death when administered concurrently with the monoclonal antibody trastuzumab [85]. In this context, ABC294640 is a sphingosine kinase 2 and dihydroceramide desaturase inhibitor whose safety and efficacy is currently being tested in an Ib/II trial in multiple myeloma refractory/relapsed patients (ClinicalTrials.gov identifier #NCT02757326). ABC294640 induces a 3-fold increase in dihydroceramides, and a concomitant decrease in c-Myc and androgen receptor, which dampens the proliferative capacity of castration-resistant prostate cancer cells [86].

6.2. Neurodegeneration

In addition to oncology, MLT is being applied to other therapeutic areas. The CNS has a very high lipid content and numerous neurodegenerative, neurological and neuropsychiatric conditions are associated with lipid alterations [53,87,88]. However, most drugs targeting CNS diseases interact with receptors or enzymes that control the activity of signaling pathways.

In this context, current Alzheimer's disease (AD) therapies with acetylcholinesterase inhibitors and NMDA receptor blockers have very limited effects on the progression of AD. In fact, only a small proportion of patients respond to these therapies, undergoing short periods of cognitive stabilization followed by a relapse in neuronal degeneration and cognitive decline [89,90]. The amyloid cascade hypothesis for the etiology of AD has greatly influenced the development of therapies. Thus, therapies against amyloid plaque formation or downstream events (e.g., tau protein phosphorylation) have been developed but they have been largely unsuccessful (see Table 1 for the clinical trials in AD targeting the amyloid cascade that were terminated during phase 2 or 3 due to lack of efficacy) [12]. These results, especially the failures with promising antibodies like Bapineuzumab and Ponezumab (ClinicalTrials.gov identifiers NCT00676143, NCT00667810, NCT00998764, NCT00996918, NCT722046 and NCT00945672: Table 1), raise the question as to whether there is another upstream event involved in the etiology of neurodegeneration.

Membrane lipids are candidates to be upstream molecules involved in the pathophysiology of AD because the structures they form in membranes (microdomains) constitute signaling platforms that receive and propagate messages into or from neurons, and in other cell types. Moreover, important alterations have been found in brain membrane lipids, making it likely that these alterations might be involved in the etiology of this condition. Indeed, brain CHO constitutes about a quarter of all the CHO in the body and CHO homeostasis appears to be involved in the pathogenesis of AD. Thus, among the lipoproteins transporting CHO to the brain, people who carry one or two copies of the ApoE4 gene isoform have a higher risk of developing AD as they less efficiently promote CHO flux in astrocytes and neurons [91]. Moreover, deregulation of CHO homeostasis is similar in patients with sporadic AD due to altered ApoE4 expression as it is when induced by presenilin-1 alterations in γ-secretase function in familial (genetic) AD models [92,93]. In addition,

Table 1
Clinical trials against Alzheimer's disease discontinued in phase 2 and 3.

Drug	Company	Mechanism of action	Clinical trial ID*(NCT)	Observations
AN-1792	Janssen Pfizer	Aβ-targeted active immunotherapy	00021723	Brain inflammation Aseptic meningoencephalitis
Avagacestat	Bristol-Myers Squibb	Notch-sparing γ-secretase inhibitor	00890890	Lack of clinical improvement Increased rate of skin cancers
Bapineuzumab	Janssen Pfizer	Aβ-targeted passive immunotherapy	00676143 00667810 00998764 00996918	Lack of clinical improvement
Lithium	Public institutions	Tau phosphorylation inhibitor	01055392 02129348 00088387	Discrepant results reported Apparently effective in early AD (amnestic MCI) but not in mild to moderate AD
LY2886721	Eli Lilly & Co.	β-Secretase inhibitor	01561430	Altered liver biochemistry
Methylthioninium	TauRx	Tau aggregation inhibitor	00684944 00515333	Discrepant results reported Blinding of phase 2 trial has been questioned
Ponezumab	Pfizer	Aβ-targeted passive immunotherapy	00722046 00945672	Lack of clinical improvement
Rosiglitazone	GlaxoSmithKline	Anti-diabetic drug Aβ clearance enhancer	00428090 00550420	Lack of clinical improvement
Scyllo-inositol	Elan Speranza Transition	Aβ aggregation inhibitor	00568776 00934050	Lack of clinical improvement
Semagacestat	Eli Lilly & Co.	γ-secretase inhibitor	01035138 00762411 00594568	Lack of clinical improvement Increased risk of skin cancer and infections.
Tarenflurbil	Myriad Genetics & Laboratories	γ -secretase modulator	00105547 00380276 00322036	Lack of clinical improvement Low potency and poor brain penetration
Tramiprosate	Neurochem	Aβ aggregation inhibitor	00314912 00088673 00217763	Lack of clinical improvement
Valproate	Abbott Laboratories	Tau phosphorylation inhibitor	00071721	Lack of clinical improvement Brain volume loss

Data obtained from ClinicalTrials.gov.

CHO and other components of lipid rafts (sphingomyelin and GM1) are able to revert insoluble amyloid peptide fibrils into soluble oligomeric forms [94].

Other lipids that are altered in the brain of AD patients are PUFAs. In fact, a reduction in DHA has been observed in the brain in association with neurodegeneration, although there are marked differences in different brain areas [95]. Moreover, lower levels of DHA have been found in lipid rafts obtained from AD patients [96], and lower levels of PE have also been found in the hippocampus of these patients [97,98]. Interestingly, this brain region is one of the areas most affected by neurodegeneration. Alterations to PE and DHA levels (as well as to CHO and sphingomyelin) cause relevant changes in the biophysical properties of AD brain membranes, including the production and interaction with membranes of amyloid peptides (the units responsible for senile plaque formation) [99,100]. Finally, treatment with 2-hydroxy-DHA (DHALifort) induces a recovery of PE and PUFA levels in the brain, and in the cognitive scores of mice expressing human AD genes [101,102]. These data further demonstrate the clear connection between membrane lipids and neurodegeneration in AD. It is likely that the alteration in membrane lipids is an event upstream of the altered APP processing that produces amyloid peptides, senile plaques, tau protein phosphorylation, neuroinflammation and other downstream alterations responsible for neuronal death in the brain of AD patients. Thus, the recovery of membrane PE and DHA levels after DHALifort treatment reduces these downstream hallmarks of AD [101,102]. This would in part explain the successful neuroprotection and neuroregeneration induced by DHALifort, and the lack of success of other drug candidates and antibodies targeting downstream events. In support of the efficacy of membrane-interacting molecules to treat AD, hydroxamic acid and dihydropyridine derivatives are known to regulate the membrane lipid nanostructure and they are HSP co-inducers with neuroprotective effects that can be used in the treatment of AD and other neurodegenerative conditions [58,103,104].

In addition to AD, other neurological conditions like Parkinson's disease (PD) or different types of sclerosis could benefit from MLT approaches. In fact, albumin-OA complex, induces significant motor recovery (~40%) in rats with spinal cord injury (SCI) [105], ameliorating both spasticity and pain. By contrast, EA, which is the trans-isomer of OA, causes no significant improvements in paralysis, spasticity or pain [105]. Moreover, the OA analogue NFX88 (Neurofix) is undergoing clinical trials for the treatment of neuropathic pain in patients with SCI. In this context, OA induces significant changes in the structure of model membranes, whereas EA has no such effect [6], indicating that the therapeutic action of the former could be mediated in part by its effects on the structure of lipids. Moreover, the lipid interacting hydroxylamine derivative arimoclomol is currently being studied to determine its efficacy against SOD1 positive familial amyotrophic lateral sclerosis in a phase 2/3 clinical trial (ClinicalTrials.gov identifier #NCT00706147).

Thus, 2-hydroxy fatty acids appear to display efficacy in treating various CNS conditions (AD, stroke, glioma, etc.), highlighting the relevant role 2-hydroxy fatty acids may have in maintaining the integrity of neural tissue. Indeed, mutation of fatty acid 2-hydroxylase (FA2H) is responsible for spastic paraplegia type 35 (SPG35), a neurological disorder that further supports the importance of 2-hydroxylated fatty acids [106] and which could be treated using these compounds.

6.3. Inflammation

Numerous lipid mediators derived from ω-3 and ω-6 PUFAs (leukotrinenes, prostaglandins, thromboxanes, eicosanoids, endocannabinoids or proresolving lipids) play a key role in inflammatory processes [107]. These compounds can modify the cell membrane composition and the activity of important transcription factors, and therefore, their analogues could be used to treat different types of inflammatory pathologies. Indeed, the arachidonic acid analogue, 2-hydroxy arachidonic acid, reduces inflammation by inhibiting the activity and expression of cyclooxygenases [108]. In addition, some alkyl lysophospholipid derivatives (e.g., miltefosine) are under development for the treatment of antihistamine-resistant urticaria. These molecules

have also been shown to be effective in treating rheumatoid arthritis, atopic dermatitis, psoriasis and allergy, most probably by regulating the structure of lipid rafts [109,110]. The structure of platelet activating factor (acetyl-glyceryl-ether-phosphorylcholine: PAF) is very similar to that of miltefosine, and it is involved in arachidonic acid metabolism and inflammation, further showing the relevance of lipids in inflammatory processes. PAF is involved in inflammation in AIDS patients and it has been suggested that the pharmacological regulation of its metabolism could reduce the mortality of these patients [111]. Furthermore, cholesteryl hemiesters cause irreversible lipidosis and inflammation [112], whereas a balanced fatty acid intake induces less lipogenic and inflammatory effects than high carbohydrate diet [113]. These studies reveal the potential of synthetic lipids and other MLT approaches to treat inflammatory processes.

Along with other therapeutic effects against cancer and neurodegenerative conditions, PUFAs have been seen to be efficacious against inflammation [114]. In this context, DHA formulated in liposomes was effective in treating chronic inflammatory diseases [115]. In addition, the MUFA, OA, has also been reported to have a positive effect against inflammation, whereas the SFA, palmitic acid, induces various types of inflammatory processes, revealing the importance of lipids in the etiology inflammation and its therapy [116,117]. Along with the enzymes and receptors with which they interact, Sphingolipids have also been associated with inflammation in vascular and respiratory tissues [118,119]. In summary, the relevant roles different lipids in various inflammatory processes suggest that they and the processes they regulate could be used as targets for anti-inflammatory drug discovery.

6.4. Cardiovascular diseases

The type and proportion of membrane lipids depends in part on lipid intake, which is associated with cardiovascular health [120,121], suggesting that lipid derivatives may play an important role in the therapy of cardiovascular diseases. In this context, erythrocyte cell membranes from elderly hypertensive subjects contain more Chol, Chol esters and triacylglycerols, and less phospholipids than age-matched normotensive controls, in association with altered G protein activity [120]. By contrast, high OA intake is associated with reduced blood pressure [120,121]. Moreover, the synthetic MUFA, 2-hydroxyoleate, induces a stronger reduction in systolic blood pressure in hypertensive rats than OA [122,123]. This dramatic reduction (70 mm Hg) in blood pressure is mediated by the regulatory effects of membrane lipids, which increase adenylyl cyclase activity, activate PKA and reduce Rho kinase expression [123]. Long chain omega-3 FA intake also induces a reduction in blood pressure [124] and while diets deficient in these FAs are associated with hypertension, dietary supplementation with alpha-linolenic acid (18:3 omega-3) diminishes high blood pressure [125]. In this context, the omega-3 FAs, EPA and DHA reduce blood pressure, and they protect against myocardial infarction and ischemic stroke [124].

As described for MUFAs, PUFA intake influences the composition of the cell membrane, which in turn regulates the structural properties of the membrane and controls the activity of membrane signaling proteins [126,127]. In addition, FAs control the transient outward K^+ current (Ito) and the transient cytosolic Ca^{2+} levels in isolated cardiomyocytes [128]. By contrast, saturated FAs and/or Chol intake has negative effects on blood pressure [129,130]. In this context, a study in a Drosophila model of atrial fibrillation also suggested that DHA derivatives may be useful to treat atrial fibrillation [131] due to their heat-stress-like effect, which influences the abundance of CHO-microdomains. Finally, 2-hydroxyarachidonic acid has also been observed to produce therapeutic benefits in a stroke animal model (see below). Moreover, atherosclerosis and stroke have also been shown to be prevented or promoted by certain lipids, the plasmatic levels of which are frequently used as a predictive biomarker for cardiovascular conditions. Thus, omega-3 fatty acids have been seen to reduce intracranial atherosclerosis and the ensuing risk of stroke [132]. By contrast, high plasma levels of CHO and triacylglycerol are associated with atherosclerosis, and a reduction of low-density lipoprotein CHO with atorvastatin impairs the progression of atherosclerosis in patients with coronary heart disease with hypercholesterolemia [133]. CHO is one of the main structural and functional constituents of cell membranes, contributing to the formation of membrane the microdomains (e.g., lipid rafts) that act as signaling platforms [50,134]. Nevertheless, high LDL/VLDL-associated CHO constitutes a major risk factor for the development of atheroma plaques as it decreases the size of the blood vessel lumen, potentially producing the ischemic thrombi that cause atherosclerosis, stroke and myocardial infarction [135,136]. Moreover, high plasmatic CHO levels are associated with increased levels of CHO in the plasma membranes of cardiovascular and other cells, altering the membrane structure, protein-lipid interactions and cell signaling [137].

In this context, statins, which inhibit CHO biosynthesis, are widely used to lower levels of plasma LDL-CHO to reduce the risk of cardiovascular disease [138]. Moreover, high CHO levels and alterations in CHO metabolism have been consistently associated with the development of AD, which exposes the relationship between cardiovascular, metabolic and neurodegenerative conditions [139]. Synthetic unsaturated FAs reduce CHO levels, and they are currently under development to treat hypercholesterolemia. Many have been developed from natural omega-3 fatty acids with known efficacy in reducing plasma CHO, either alone or in combination with statins [140]. In fact, dietary omega-3 FAs are associated with blood pressure reduction and are considered to be beneficial for prevention of hypertension [141,142].

6.5. Metabolic diseases

Obesity is an important health disorder, the prevalence of which has reached epidemic proportions in industrialized countries. Consumption of the cis-MUFA, OA (omega-9), is associated with lower body mass index (BMI) values [143]. Moreover, in rats with ad libitum access to food, a daily supplement of olive oil (in which OA constitutes about 70–80% of all FAs) induces reductions in body weight [144]. By contrast, the *trans* isomer of OA, EA does not produce any loss in body weight. OA and EA have the same chemical composition but their different molecular structures have divergent effects on membrane structure [6]. Hence, the regulation of membrane lipid structure, and the ensuing changes in protein-lipid interactions could be responsible for the therapeutic effects of OA and its derivatives, or the health problems associated with trans-MUFA consumption [120,144,145]. Saturated (or trans-MUFA) fats have been linked with obesity and related health problems. Moreover, the saturated FA palmitic acid is a known inducer of endoplasmic reticulum (ER) stress and cell death [146]. Indeed, lipstatin (a potent natural inhibitor of pancreatic lipases) and its derivative orlistat (a FASN inhibitor and lipid absorption blocker), are effective in the treatment of obesity [147].

In this context, OA analogues induce significant reductions in body weight in rats by specifically acting on fat depots [121,144]. The effect of 18-carbon FAs on body weight is highly structure-based, hydroxyoleate having the strongest effect (an 11.4% reduction after a 7-day treatment). The molecular mechanism of action of hydroxyoleate is associated with two well-documented pathways. First, it induces overexpression of the uncoupling proteins UCP1 (~3000%) and UCP3 (~350%) [144]. Like mitochondrial ATPase, UCPs channel protons from the intermembrane space to the mitochondrial matrix, which generates heat instead of ATP synthesis. This loss of energy accounts for approximately 50% of the body weight reduction observed in rats. Secondly, hydroxyoleate treatment results in a decrease in food intake, which accounts for the remaining weight loss given that the body weight reduction observed in untreated pair-fed animals is 50% of that seen in hydroxyoleate-treated animals [144].

The increased incidence of diabetes in developed countries has paralleled that of obesity in recent years [148–150]. Numerous studies

demonstrate the association between the type of dietary fats consumed and the development of diabetes [151,152]. Various studies have shown that patients with type 2 diabetes have higher saturated-to-cis-unsaturated FA ratios in membranes from various tissues with respect to healthy controls [153–155]. Thus, high OA intake improves the glycemic status of these patients and it also reduces the levels of SFAs, while increasing those of cis-unsaturated FAs, provoking a reduction in the saturated-to-unsaturated FA ratio [153]. In contrast to cis-MUFAs, the consumption of *trans* unsaturated fats, the structure and effects of which on membrane structure are closer to those of saturated fats, is also associated with diabetes and other health problems [156]. In this scenario, treatments with cis-unsaturated FA and their derivatives are potentially useful against diabetes [145,153,157,158]. Furthermore, the modulator of membrane structure and HSR activator, BGP-15, could potentially be used to treat patients with type 2 diabetes, and its efficacy and safety are currently under study (ClinicalTrials.gov identifier #NCT1069965).

7. Future of membrane lipid therapy

The development of drugs targeting membrane lipids has tremendous potential and interest in thia approach has increased significantly of late. In the past century, drug treatments aimed to regulate protein activity, or to a lesser extent, nucleic acids. In this scenario, lipids were only considered to be relevant players in metabolic diseases. However, our current knowledge of the important roles of membrane lipids in health and disease makes it possible to define new therapeutic approaches involving the regulation of membrane lipids. A combination of treatments targeting lipid bilayers and an increasing knowledge of the role of membranes will likely yield many more drug treatments based on MLT in near future. This volume brings together a series of reviews and papers on key aspects of MLT. The influence of **membrane lipids in the cell's activity** has been studied in some of the works in this Special Issue. Thus, Poveda et al. reviewed the interactions and effects of membrane lipids with ion channels, showing their importance in the structure and function of these transmembrane proteins [159]. Indeed, membrane channels can be regulated by lipids, either through direct binding or by modulation of the lipid bilayer's biophysical properties. These direct and indirect effects can modify the localization and activity of TRP ion channels, which could be associated with various pathologies, as described elsewhere in this volume [160]. Other transmembrane proteins appear to be crucial to its pathophysiology, such as SMS1, the structure of which is reported in this issue by Piotto et al. [161]. Lipid-lipid and protein-lipid interactions in membranes were studied by Casas et al., who showed that the lipid moieties of amphitropic G proteins that interact with membranes regulated membrane lipid structures, and controlled G protein-membrane interactions with specific membrane microdomains [162]. Interestingly, Gβγ dimers and Gαβγ trimers bear an isoprenyl moiety that facilitates the formation of nonlamellar phases in vitro, which in turn enhances the binding of both types of oligomeric G proteins to the membrane. By contrast, the fatty acyl moieties in Gα preserve the lamellar propensity of lipid bilayers, helping this monomer bind to this type of microdomain [162]. These results clearly indicate that protein-lipid interactions at the membrane are bidirectional. Not only do lipids affect the binding of proteins to membranes but protein binding to membranes also modulates membrane lipid structure, which in turn establishes a cooperative effect in protein-lipid interactions. Moreover, Noguera-Salvà et al. unraveled the role of positively charged amino acids and hydrophobic C-terminal modifications (C-terminal proteolysis, methylation and isoprenylation) on $G\gamma_2$ protein for cell membrane subcellular localization [163]. The role of **lipids in the cell's physiology** was addressed by van der Veen et al., who reviewed the importance of PC and PE in the plasma and organelle membranes, and the impact of the PC:PE ratio in health and disease [164]. Karunakaran and van Echten-Deckert reviewed the role of sphingosine 1-phosphate in brain cell signaling and its involvement in disease and therapy [165]. The role of lipin phosphatidic acid phosphatases as a branch point for the synthesis of triacylglycerols or phospholipids, and the ability of these enzymes to transit among different cell membranes, was addressed by Zhang and Reue [166]. Alterations in the levels of different lipins, or mutations in the genes encoding these enzymes, have been seen to cause conditions with a variety of symptoms, indicating their relevance in different activities and the possibility of developing therapies to regulate lipins and the lipids they control. Martínez-Gardeazabal et al. presented a rat brain lipid map using imaging mass spectrometry. As a result, they show that lipid alterations can be used to characterize neurological disorders and that they might be involved in the etiology of CNS conditions [167].

Finally, **therapeutic approaches involving MLT, using either natural or synthetic lipids** were highlighted in many of the works published in the present volume. One of the main areas for the development of membrane lipid-based therapies is the CNS, which contains a very large proportion of lipids. Accordingly, MLT therapies appear as potential alternatives for the treatment of AD, showing that DHA-H can induce the recovery of cognitive functions in a Drosophila model of this human neuropathology [168]. Moreover, González-de San Román et al. demonstrated the relevance of lipids in the pathophysiology of AD, which further indicates their utility in neurodegeneration therapy [169]. Lipids are also related to pain and its therapy, as highlighted by Ferrer-Montiel and Ciardo and by Galan-Arriero et al. [160,170]. In this context, brain membrane lipids have been also shown to be involved in the etiology of stroke and treatment with the arachidonic acid analog, 2OAA, markedly prevented neuronal death in the brain [171]. Stroke is the first or second cause of death in most industrialized countries and the use of the synthetic lipid 2OAA for its therapy brings new options for a condition with important unmet clinical needs.

Interestingly, lipids are involved in other pathologies and their therapy, including: infectious diseases [172], cancer [173,174], dermatological pathologies [175], metabolic disorders [176], fatigue [177], respiratory conditions [178,179], etc. In fact, lipid replacement therapy, which aims to restore membranes through the administration of mixtures containing specific glycerophospholipids and fructooligosaccharides, has proven efficacious in the treatment of cancer and aging [177]. In summary, among the pleiotropic effects that pathological alterations induce, lipid dysregulation constitutes a relevant factor in the etiology of many diseases. Therefore, MLT strategies could be applied to many patients, especially in fields with important unmet clinical needs that are calling out for innovative therapeutic approaches.

Conflict of interest

The author has no conflict of interest to declare.

Transparency document

The Transparency document associated with this article can be found in the online version.

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