



## Bioactive Lipids and Their Derivatives in Biomedical Applications

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### Abstract

Lipids, which along with carbohydrates and proteins are among the most important nutrients for the living organism, have a variety of biological functions that can be applied widely in biomedicine. A fatty acid, the most fundamental biological lipid, may be classified by length of its aliphatic chain, and the short-, medium-, and long-chain fatty acids and each have distinct biological activities with therapeutic relevance. For example, short-chain fatty acids have immune regulatory activities and could be useful against autoimmune disease; medium-chain fatty acids generate ketogenic metabolites and may be used to control seizure; and some metabolites oxidized from long-chain fatty acids could be used to treat metabolic disorders. Glycerolipids play important roles in pathological environments, such as those of cancers or metabolic disorders, and thus are regarded as a potential therapeutic target. Phospholipids represent the main building unit of the plasma membrane of cells, and play key roles in cellular signaling. Due to their physical properties, glycerophospholipids are frequently used as pharmaceutical ingredients, in addition to being potential novel drug targets for treating disease. Sphingolipids, which comprise another component of the plasma membrane, have their own distinct biological functions and have been investigated in nanotechnological applications such as drug delivery systems. Saccharolipids, which are derived from bacteria, have endotoxin effects that stimulate the immune system. Chemically modified saccharolipids might be useful for cancer immunotherapy or as vaccine adjuvants. This review will address the important biological function of several key lipids and offer critical insights into their potential therapeutic applications.

**Key Words:** Lipids, Fatty acids, Glycerolipids, Phospholipids, Biomedical applications

### INTRODUCTION

Lipids are chemically defined as hydrophobic molecules that are insoluble in water but soluble in apolar solvents. In the biological context, lipids are essential molecules that function as the major structural components of living cells and play important roles in various cellular functions (Fahy *et al.*, 2011). For many years, lipids were known only as energy-storing molecules and the building blocks of cellular membranes. However, with the progress of advanced analytical techniques and bioassays, additional classes and functions of lipids are increasingly coming to light. Today, lipids are known to be involved in numerous cellular signaling pathways, including those that regulate cell proliferation, survival, apoptosis, and cell metabolism (Alabdulkarim *et al.*, 2012). Due to their excellent biocompatibility, lipids are also used as efficient and safe carriers for drug delivery in various formulations (Yang and Merlin, 2020).

Lipids are classified not only as hydrophobic compounds but also as amphiphilic molecules with both hydrophilic and hydrophobic properties. This property of lipid molecules means that they arrange themselves in the biophysical environment as bilayer structures (also called “micelles”) wherein the hydrophobic part is sequestered to the interior and the hydrophilic part is exposed to the aqueous environment. This self-organization determines the morphology and functions of lipids in living organisms. For example, bilayers of phospholipids comprise the cellular and subcellular membranes of living cells. Their amphiphilic properties govern their interactions with transmembrane proteins and modulate their activity (Watson, 2015). Lipids may also be located in droplets stored within cells (Welte and Gould, 2017) or circulate in blood as lipoprotein complexes (Fernandez *et al.*, 2013). Glycolipids play important roles in the immune system (Zhang *et al.*, 2019a).

Dysregulated lipid levels have been reported in various diseases, such as cancer, obesity, diabetes, and cardiovascular,

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autoimmune, neurodegenerative diseases (Leuti *et al.*, 2020). This, combined with their important roles in various biological conditions, suggests that lipids might be potential therapeutic targets in disease treatment. In this review, we categorize lipids based on their structures and relevant biological activities (Fig. 1), update and summarize the recent applications of bioactive lipids in disease treatment and biomedicine (Fig. 2), and discuss nano/micro formulations that have improved the therapeutic effects of bioactive lipids.

## FATTY ACIDS

Fatty acids are hydrocarbon derivatives that have a carboxylic acid with a long aliphatic chain (Kimura *et al.*, 2020). Generally, naturally occurring aliphatic chains of fatty acids have 4-28 carbon atoms connected by single or double bonds. They can be classified as saturated fatty acids, which have only single carbon-carbon bonds in the aliphatic chain, or unsaturated fatty acids, which have one or more double bonds in the aliphatic chain (Currie *et al.*, 2013). Examples of biomedical applications of fatty acids are listed in Table 1.

### Short-chain fatty acids

Short-chain fatty acids have aliphatic chains with fewer than six carbon atoms and play important roles in immune cell activity and modulation of G protein-coupled receptor signaling (Kim, 2021; Moniri and Farah, 2021). Short-chain fatty acids are produced by the anaerobic fermentation of dietary fibers in the intestine (Koh *et al.*, 2016). They exert their biological activity via inhibition of histone deacetylase (HDAC); this activity is not only confined to the intestinal area but is also detected in the blood, leading to systemic effects (Parada Venegas *et al.*, 2019). Gut microbiota-derived short-chain fatty acids, such as acetic acid (C2:0), propionic acid (C3:0), and butyric acid (C4:0), are key immune system-regulating metabolites and their dysfunction is related to inflammatory disease (Lavelle and Sokol, 2020).

Propionic acid which is a major short-chain fatty acid produced from gut microbiota fermentation, protects against allergic inflammation in the lung (Trompette *et al.*, 2014). Propionic acid acts as a ligand of G protein-coupled receptor 41 (GPR41) and G protein-coupled receptor 43 (GPR43) (Husted *et al.*, 2017). An immunomodulatory effect of propionic acid was also observed in human cells. Based on the bioactivity of propionic acid, it has been studied for the treatment of autoimmune diseases, such as multiple sclerosis (Duscha *et al.*, 2020). After supplementation of propionic acid for 14 days, the gut microbiome of multiple sclerosis patients was altered and the imbalance between regulatory T cells (Tregs) and helper T cell type 17 (Th17) in the central nervous system was improved. As a result, the subcortical gray matter of the patients was increased. These findings support the potential clinical application of propionic acid.

Butyric acid is another widely studied short-chain fatty acid. Similar to propionic acid, butyric acid shows anti-inflammatory activity. It plays important roles in maintaining the homeostasis of commensal bacteria through the regulation of intestinal macrophages (Chang *et al.*, 2014). Butyric acid was reported to induce anti-inflammatory characteristics in bone marrow-derived macrophages and colonic lamina propria macrophages. Mechanistically, butyric acid reduced the lipopolysac-

charide (LPS) stimulation-induced secretions of nitric oxide, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and IL-6 through histone deacetylase inhibition, but not via the G-protein coupled receptor. Despite these anti-inflammatory effects, however, butyric acid treatment did not ameliorate colitis in dextran sulfate sodium (DSS)-treated model mice due to a lack of sufficient production of protective cytokines, such as IL-6.

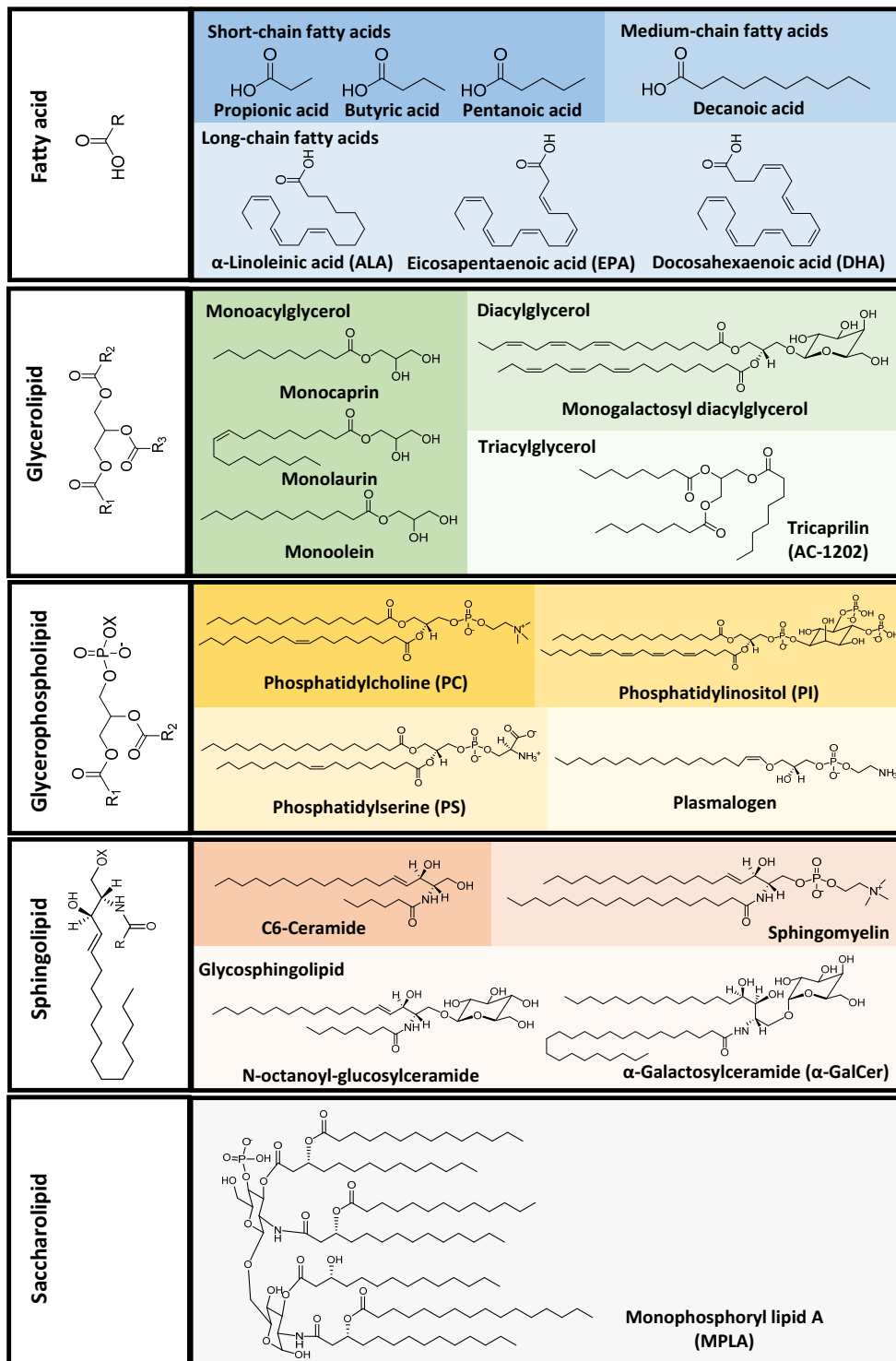
Other studies have shown that butyric acid may have therapeutic potential for inflammatory bowel disease due to its ability to modulate epithelial function or gut microbiota (Zheng *et al.*, 2017; Dou *et al.*, 2020; Facchin *et al.*, 2020; Wang *et al.*, 2020). Butyric acid, but not other short-chain fatty acids, up-regulated the actin-binding protein, synaptopodin, at the intestinal epithelial tight junction, which is important for epithelial barrier function and wound healing. Moreover, butyric acid supplementation alleviated the phenotypes in synaptopodin-deleted mice experiencing DSS-induced colitis (Wang *et al.*, 2020). In terms of modulating the gut microbiota, orally administered sodium butyric acid showed therapeutic efficacy in the DSS-induced colitis mouse model and increased the populations of Lachnospiraceae and Lachnospiraceae (Dou *et al.*, 2020), and administration of butyric acid was reported to change the gut-microbiota environment in inflammatory bowel disease patients (Facchin *et al.*, 2020). In the latter work, oral administration of microencapsulated sodium butyric acid improved the gut microbiota composition of ulcerative colitis and Crohn's disease patients, as evidenced by alterations in short-chain fatty acid-producing microorganisms (Lachnospiraceae spp. and Butyricoccus, respectively). Sodium butyric acid also reportedly improved the quality of life among ulcerative colitis patients.

Pentanoic acid (C5:0, also called valeric acid) has also been reported as an immunoregulatory short-chain fatty acid that might be a potential therapeutic for autoimmune disease (Luu *et al.*, 2019). Pentanoate elevated the glucose oxidation of lymphocytes via mTOR activation to suppress IL-17A expression and enhance IL-10 expression. Consequently, in experimental colitis and multiple sclerosis mouse models, pentanoate ameliorated the disease via regulatory B cell-mediated protection.

### Medium-chain fatty acids

Medium-chain fatty acids have six to 12 carbon atoms in their aliphatic chains; they represent a unique form of dietary fat and show variable biological functions (Kimura *et al.*, 2020). Physicochemical features of fatty acids have been reported to be affected by the hydrocarbon tail lengths (Ramirez *et al.*, 2001; Kimura *et al.*, 2020). The decrease of the hydrocarbon tail lengths is known to increase the water solubility of fatty acids. Thus, compared to long-chain fatty acids, medium-chain fatty acids show increased water solubility, resulting in higher oral absorption (Chen *et al.*, 2021). Medium-chain fatty acids are transported directly to the liver without requiring the carnitine carrier system, and are therefore rapidly metabolized and converted to fuel. After medium-chain fatty acids are degraded via  $\beta$ -oxidation, they produce large amounts of acetyl-CoA and ketone bodies, such as acetone, acetoacetate and  $\beta$ -hydroxybutyric acid (McGarry and Foster, 1980).

Ketone bodies are utilized as an energy source for the heart, brain, and skeletal muscles. They also have other biological functions: For example, ketone bodies reportedly control the electrical activity of neurons by regulating transporters

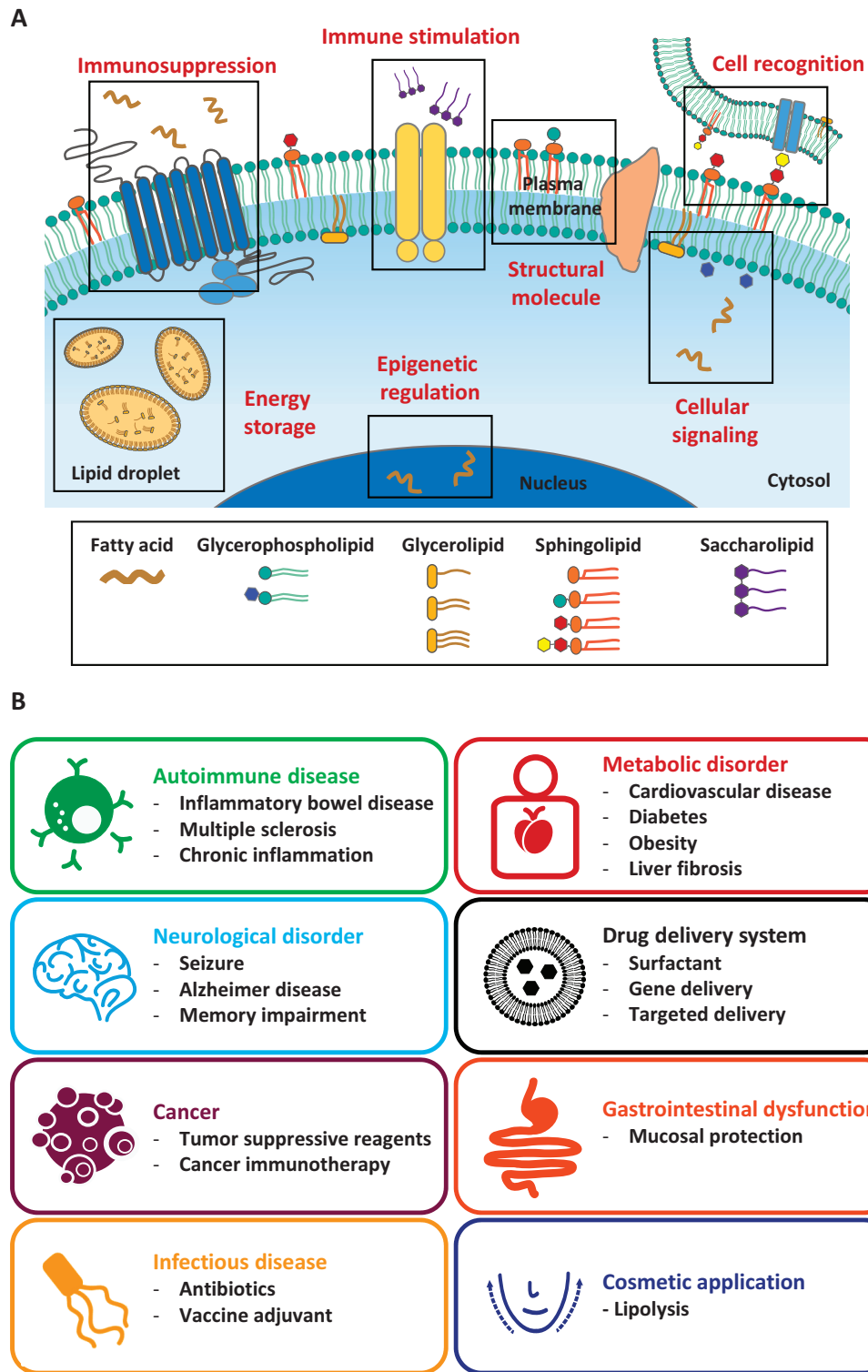


**Fig. 1.** Structure-based classification of lipids and representative lipid molecules for biomedical application.

(e.g., vesicular glutamate transporters) and ion channels (e.g., ATP-sensitive  $K^+$  channels and voltage-dependent  $Ca^{2+}$  channels) (Juge *et al.*, 2010; Tanner *et al.*, 2011; Kadowaki *et al.*, 2017). In the therapeutic context, ketone bodies and ketone body-generating medium-chain fatty acids may affect neuro-

logical diseases, and a medium-chain triglyceride-based ketogenic diet has been applied for seizure control since 1920s (Warren *et al.*, 2018).

Although seizure control requires ketone bodies originating from medium-chain fatty acids, several studies found



**Fig. 2.** Biomedical activities and applications of lipids. (A) Lipids play a wide range of biological roles in regulating cell homeostasis through various processes, including cell structure formation, energy storage, and cellular signaling. (B) Biomedical applications of lipids in human medicine.

that medium-chain fatty acids themselves might directly control neuronal activity (Sada and Inoue, 2018). Decanoic acid (C10:0), which is a monocarboxylic saturated fatty acid with 10

carbon atoms, inhibited the activation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits stimulated by glutamate-induced currents, showing a greater

**Table 1.** Biomedical activity and application of fatty acids

Class	Name	Activity	Application	Ref.
Short-chain fatty acids	Propionic acid	Immunomodulatory effect on mature dendritic cells	Anti-inflammatory activity	Nastasi <i>et al.</i> , 2015
		Enhance regulatory T cell metabolism and function	Multiple sclerosis	Duscha <i>et al.</i> , 2020
		Alteration of bone marrow hematopoiesis	Allergic airway disease	Trompette <i>et al.</i> , 2014
	Butyric acid	Microencapsulated butyric acid for regulation of microbiota (ButyRose)	Inflammatory bowel disease	Facchin <i>et al.</i> , 2020
		Enhanced epithelial barrier function through IL-10 receptor	Inflammatory bowel disease	Zheng <i>et al.</i> , 2017
		Alleviates mouse colitis by regulating gut microbiota dysbiosis	Inflammatory bowel disease	Dou <i>et al.</i> , 2020
		Regulation of intestinal homeostasis through actin-associated protein synaptopodin	Inflammatory bowel disease	Wang <i>et al.</i> , 2020
		Intestinal macrophage regulation via histone deacetylase inhibition	Inflammatory bowel disease	Chang <i>et al.</i> , 2014
	Pentanoic acid	Inducing IL-10 production in lymphocytes by reprogramming their metabolic activity	Experimental autoimmune encephalomyelitis	Luu <i>et al.</i> , 2019
Medium-chain fatty acids	Decanoic acid	Synergistic action against AMPA receptors and seizures with perampanel	Seizure control	Augustin <i>et al.</i> , 2018a
		Inhibition of glutamate-induced currents derived from various types of AMPA receptor	Seizure control	Chang <i>et al.</i> , 2016
Long-chain fatty acids	$\alpha$ -linolenic acid	Promoting bioconversion and subsequent oxylipin formation	High fat diet induced Insulin resistance	Fan <i>et al.</i> , 2020
		Enhanced production of oxylipins in M1 macrophage	Anti-inflammatory activity	Pauls <i>et al.</i> , 2018
		Activation of alternatively activated macrophages via oxylipin profile change	Anti-inflammatory activity	Pauls <i>et al.</i> , 2020
		Modulation of phagocytosis and endosomal pathways of extracellular Tau in microglia	Alzheimer's disease	Desale and Chinnathambi, 2021
	Eicosapentaenoic acid	Alleviates dextran sulfate sodium-induced ulcerative colitis in mice	Inflammatory bowel disease	Kim <i>et al.</i> , 2020
		Improvement of hepatic metabolism and reduces inflammation in mice and HepG2 cells	Nonalcoholic fatty liver disease	Albracht-Schulte <i>et al.</i> , 2019
	Docosahexaenoic acid	Inhibition NLRP3 inflammasome activation	Acute cerebral infarction (ACI)	Mo <i>et al.</i> , 2020
Enhanced M2 macrophage polarization via the p38 signaling pathway and autophagy		Chronic inflammation	Kawano <i>et al.</i> , 2019	
		Modulate monocyte inflammatory response	Chronic inflammation	So <i>et al.</i> , 2021

impact on the GluA2/3 subunit versus the GluA1 or GluA1/2 subunits (Chang *et al.*, 2016). Decanoic acid also showed a synergistic interaction with the current epilepsy drug, perampanel, in antagonizing AMPA receptors and treating seizures (Augustin *et al.*, 2018a).

### Long-chain fatty acids

Long-chain fatty acids have 12 or more carbon atoms in their aliphatic chains; they are generally synthesized in animal or plant cells and generate energy via their  $\beta$ -oxidation-mediated degradation to acetyl-CoA. Even-number saturated fatty acids, such as palmitic acid (C16:0) and stearic acid (C18:0), are the predominant fatty acids used for energy stor-

age in animal tissues (Kimura *et al.*, 2020). However, some unsaturated long-chain fatty acids have other biological functions, as well as pharmacological activities that may be useful for therapeutic applications (Valenzuela *et al.*, 2021).

Polyunsaturated long-chain fatty acids are particularly important signaling molecules for physiological homeostasis. Polyunsaturated long-chain fatty acids can be classified based on the position of their double bond (Simopoulos, 2016). Omega-6 fatty acids, which have a double bond located at six carbon atoms away from the terminal methyl end of their backbone, are synthesized from linoleic acid (LA, C18:2n-6), which is an essential fatty acid that must be consumed from food as human body cannot synthesize (Simopoulos, 2016).

Omega-3 fatty acids have a double bond located at three carbon atoms away from the terminal methyl end of their aliphatic chain, and are synthesized from the essential fatty acid,  $\alpha$ -linolenic acid (ALA, C18:n-3). Omega-6 fatty acids generally show pro-inflammatory activity, while omega-3 fatty acids show anti-inflammatory activity (Serhan *et al.*, 2008; Serhan and Chiang, 2013). As a result, omega-3 fatty acids can show beneficial effects in chronic inflammatory disease or metabolic disorders (Valenzuela *et al.*, 2021). GRP40 and GRP120 have been identified as receptors of omega-3 and omega-6 fatty acids (Oh *et al.*, 2010).

ALA is considered to have therapeutic potential. It is a precursor of oxylipins, such as eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-22), which are oxygenated metabolites that have anti-inflammatory activities (Serhan *et al.*, 2008; Serhan and Chiang, 2013). At the cellular level, ALA has been reported to regulate macrophages via oxylipin production, as evidenced by reduced LPS-triggered secretion of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  by macrophages and increased phagocytic activity and altered production of the chemokine, monocyte chemoattractant protein-1, by M2-like cells (Pauls *et al.*, 2018, 2020). ALA-induced enhancement of migratory ability increases phagocytosis and endosomal processing of extracellular Tau in microglia (Desale and Chinnathambi, 2021). ALA has shown anti-inflammatory activities in animal models of metabolic or inflammatory diseases. For example, ingestion of ALA-enriched butter elevated the blood level of eicosapentaenoic acid and suppressed the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B), and these changes were associated with improvement of insulin resistance and tissue inflammation in high fat diet-fed mice (Fan *et al.*, 2020). ALA treatment also halted colon contraction and improved clinical symptoms in the DSS-induced colitis mouse model (Kim *et al.*, 2020).

Eicosapentaenoic acid and docosahexaenoic acid-derived mediators (such as resolvins, protectins, and maresins) can contribute to the resolution of inflammatory conditions and are

thus termed “pro-resolving” mediators (Serhan *et al.*, 2008; Bäck *et al.*, 2019). Eicosapentaenoic acid is oxidized to E-series resolvins (RvE1, RvE2, and RvE3) by cyclooxygenase and lipoxygenase. RvE1 and RvE2 are endogenous antagonists of the leukotriene B<sub>4</sub> (LTB<sub>4</sub>) receptor, BLT-1, and can thereby block the chemotaxis of polymorphous-nuclear neutrophils and activation of NF- $\kappa$ B. These resolvins also activate ChemR23/ERV, which regulates pro-inflammatory cytokine production (Spite *et al.*, 2014). Eicosapentaenoic acid was further reported to exert anti-inflammatory activity by inhibiting nucleotide binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3) and to show therapeutic efficacy in acute cerebral infarction or nonalcoholic fatty liver disease (Albracht-Schulte *et al.*, 2019; Mo *et al.*, 2020). Docosahexaenoic acid may be metabolized to D-series resolvins (RvD1-6), protectins (PD1/NPD1), maresins, and RvD1, which shares working mechanisms similar to those of RvE1 and RvE2 (Serhan and Chiang, 2013). Similar to eicosapentaenoic acid, docosahexaenoic acid has been demonstrated to act as an anti-inflammatory molecule that regulates macrophages and monocytes (Kawano *et al.*, 2019; So *et al.*, 2021).

## GLYCEROLIPIDS

A glycerolipid is classified as a monoacylglycerol (MAG), diacylglycerol, or triacylglycerol (TAG) based on how many ester bonds are formed between the fatty acid with the glycerol backbone. Glycerolipids can be found in the membranes of prokaryotes and eukaryotes and in animal fat tissues, and are usually considered to act as an energy reservoir. The hydrolysis of the ester linkage between glycerol and fatty acid is the initiating step of fat metabolism. Many types of glycerolipids are found in human tissues, with some having been shown to have therapeutic effects (Table 2).

**Table 2.** Biomedical activity and application of glycerolipids

Subclass	Name	Activity	Medical application	Ref.
Monoacylglycerol	Monocaprylate	Formation of transient or permanent pores in the membrane	Antimicrobial	Hylgaard <i>et al.</i> , 2012
	Monolaurin	Bactericidal activity against <i>H. pylori</i>	Infectious disease	Bergsson <i>et al.</i> , 2002
	Monocaprin			
	Monoolein	Antimicrobial activity against <i>S. aureus</i> and <i>E. coli</i>	Antimicrobial	Jumina <i>et al.</i> , 2019
	MAG-DHA	Downregulation of NF- $\kappa$ B and upregulation of PTEN	Non-small cell lung cancer	Morin and Fortin, 2017
Diacylglycerol	Monogalactosyl diacylglycerol	Inhibition of CD31 positive tumor blood vessel growth.	Colon cancer	Maeda <i>et al.</i> , 2013
	1,3-Diacylglycerol	Increasing oxidation of fatty acid and suppressing chylomicron formation	Obesity and insulin resistance	Saito <i>et al.</i> , 2007
Triacylglycerol	Medium-chain triglyceride	Suppression of IL-6, iNOS, cyclooxygenase-2 and IL-10 and activation of NF- $\kappa$ B and p38 MAPK pathways	Insulin resistance	Geng <i>et al.</i> , 2016
	Medium-chain triglyceride	Metabolized to ketone bodies to the brain	Neurological and metabolic disorders	Augustin <i>et al.</i> , 2018b
	Tricaprilin (AC-1202)	Reducing oxidative damage and improvement of respiration rate in brain	Alzheimer’s disease	Henderson <i>et al.</i> , 2009

### Monoacylglycerols

Monoacylglycerols, which can exhibit different forms depending on the site at which the fatty acids form ester bonds with a hydroxyl group of glycerol, are generated by enzymatic hydrolysis of triacylglycerols or diacylglycerols. Monoacylglycerols and diacylglycerols are often used as food emulsifiers. Integration of a monoacylglycerol into the membrane of microbial cells is a common means to disrupt the membrane or induce cell lysis (Hyldgaard *et al.*, 2012). The electron transport chain and oxidative phosphorylation play important roles in energy production for bacteria. Monoacylglycerols disrupt the electron transport chain by binding to electron carriers and block oxidative phosphorylation by reducing the membrane potential and proton gradient. Among the monoacylglycerols, monocaprin and monolaurin showed the strongest bactericidal effects on *Helicobacter pylori* (*H. pylori*) (Bergsson *et al.*, 2002).

### Diacylglycerols

There are two forms of diacylglycerol: 1,2-diacylglycerols and 1,3-diacylglycerols. Diacylglycerols are used commercially as surfactants and fat emulsifiers and, within the cell, act as intermediates and cellular messengers. Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>) bound to the cellular membrane can be hydrolyzed into diacylglycerol, and this plasma membrane-localized diacylglycerol can activate protein kinase C. In T cells, this activation stimulates transcription factors, such as NF- $\kappa$ B and AP-1, to activate T cell receptor. Diacylglycerols also play important roles in activating immune cells, and several studies have tried to utilize diacylglycerol for immunotherapy. For example, researchers inhibited diacylglycerol kinase (DGK), which is the negative regulator of diacylglycerol-mediated signaling, to enhance the anti-cancer effect of natural killer (NK) cells. The same authors also observed that knocking out DGK $\zeta$  in NK cells enhanced downstream receptor activation (Yang *et al.*, 2016). Another study found that inhibition of DGK amplified the diacylglycerol signaling cascade of T cells (Arranz-Nicolás *et al.*, 2018).

Chimeric antigen receptor-T cells showed higher expression of DGK $\alpha/\zeta$  compared to spleen-derived wild type cells, which has been proposed as a strategy to advance chimeric antigen receptor-T cell immunotherapy (Moon *et al.*, 2014). Oral consumption of spinach-derived monogalactosyl diacylglycerol showed a tumor-suppressive effect in a murine model: Colon tumor (Colon 26)-inoculated groups fed with 4 mg/kg or 20 mg/kg exhibited decreases in the tumor load compared with the vehicle control (Maeda *et al.*, 2013). Diacylglycerols have also shown promising therapeutic effects against metabolic disorders. High fat diet-induced obese and brown adipose tissue-deficient mice were fed with diacylglycerol oil or triacylglycerol oil for 15 weeks. Mice fed diacylglycerol oil showed a slight decrease in body weight but an increase in glucose intolerance, compared to the triacylglycerol oil-fed group (Saito *et al.*, 2007). Finally, a study showed that accumulated hepatic diacylglycerol activated PKC $\epsilon$ , which phosphorylated insulin receptor kinase at T1160 to inhibit IRK activation and cause insulin resistance (Lyu *et al.*, 2018). However, the importance of diacylglycerol in lipid-induced hepatic insulin resistance remains controversial.

### Triacylglycerols

Triacylglycerols are tri-esters composed of glycerol bound

to three fatty acid molecules, and can be subcategorized into saturated and unsaturated groups based on the presence of double bonds in the fatty acid chain. They are commonly used as an index for metabolic syndrome. Orally applied medium-chain triglycerides have been examined as potential therapeutics against metabolic disorders and inflammation. Inflammatory signals are known to activate JNK and IKK and target insulin receptor substrate to inhibit the insulin signaling cascade. Oral application of medium-chain triglycerides was found to alleviate insulin resistance in high fat diet-induced obese mice by reducing the inflammatory response in obesity. Mice fed with medium-chain triglycerides also gained less body weight, and eventually lost up to 15% of their body weight (Geng *et al.*, 2016).

The triglyceride-glucose index is used as a marker for symptomatic coronary artery disease and atherosclerosis (Da Silva *et al.*, 2019); high triglyceride-indexed patients tend to have artery stiffness and coronary artery calcification (Won *et al.*, 2018). A medium-chain triglyceride-based ketogenic diet is becoming a prevalent trend for treating metabolic disorders. Moreover, recent studies have focused on applying medium-chain triglycerides to treat Alzheimer's disease. The brain uses two main types of fuel in its metabolic pathways: ketone and glucose. At an early stage of Alzheimer's disease, cerebral glucose hypometabolism is observed, and cells metabolize ketones rather than glucose.

An orally dosed triacylglyceride is hydrolyzed into medium-chain fatty acids by lipases in the gastrointestinal tract; these fatty acids are absorbed and metabolized through  $\beta$ -oxidation, leading to the production of ketone bodies ( $\beta$ -hydroxybutyric acid, acetoacetate, and acetone) (Augustin *et al.*, 2018b). Different kinds of triacylglycerols are undergoing clinical trials in Alzheimer's disease patients. One example is the orally administrable ketogenic compound, AC-1202, which is a medium-chain triglyceride composed of glycerin and caprylic acid. AC-1202 was shown to increase the serum level of the ketone body,  $\beta$ -hydroxybutyric acid, and significantly increase the cognitive subscale of the Alzheimer's Disease Assessment Scale compared with placebo (Henderson *et al.*, 2009).

## GLYCEROPHOSPHOLIPIDS

Glycerophospholipids and phospholipids are the main lipid constituents of the eukaryotic cellular membrane. In general, phospholipids are amphiphilic molecules with structures containing a polar portion and a non-polar portion (van Meer *et al.*, 2008). Glycerophospholipids share the basic structure of a glycerol backbone conjugated with hydrophobic acyl chains, plus a phosphate group and a hydroxyl group. The chemical structures of phospholipids vary based on the head polar groups, the properties (number, length, and saturation) of the hydrophobic acyl chains, and the type of bond formed between the hydrophobic chain and the glycerol (Li *et al.*, 2015).

Phospholipids can be grouped into five classes based on the polar head group: phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine (Harayama and Riezman, 2018). The molecular species in each class vary relative to the acyl chains at the sn1 and sn2 positions. In general, a saturated or mono-saturated fatty acid is located at the sn1 position and a poly-unsaturated fatty acid with a longer acyl chain is located at the

sn2 position (Zarringhalam *et al.*, 2012).

Phospholipids are among the most basic but important compounds that contribute to life activities. They are found ubiquitously in humans, animals, plants, and bacteria, and are considered to be the bare bones of cellular and sub-cellular membranes, in which they assemble as a bilayer (van Meer *et al.*, 2008). Phospholipids contribute to the formation of circulating lipoproteins, which act as carriers to transport hydrophobic triglycerides and cholesterol through the bloodstream (Boffa and Koschinsky, 2019). Phospholipids also act as a natural emulsifier in the body, such as by emulsifying fat-soluble substances in the presence of cholesterol and bile acid in the gallbladder, to facilitate the absorption of these substances (Ko *et al.*, 2020). They function as pulmonary surfactants to reduce the surface tension of the air/liquid interface in tissues, such as the pleura and alveoli of the lung (Agassandian and Mallampalli, 2013). Finally, phospholipids may act as signaling mediators for many important cellular functions (van Meer *et al.*, 2008). Examples of bioactive glycerophospholipids are listed in Table 3.

**Phosphatidylcholine (PC)**

PC is a main component of lecithin. Classified as glycerophospholipid, PC is composed of choline as its polar head,

fatty acids as its hydrophobic domains, and glycerol as their connector. The fatty acids of PC generally include one mono-unsaturated fatty acid and one saturated fatty acid. PC dominates among the phospholipids that comprise the cell membrane, particularly in the exoplasmic leaflet of the plasma membrane (Harayama and Riezman, 2018). PC has excellent biocompatibility and safety, as it originated from nature, and has long been approved as a major ingredient in various commercial liposome formulations (Barenholz, 2012). Beyond its use as a component of lipid-based carriers, PC can also perform potential therapeutic activities, such as inducing lipolysis, reducing inflammatory responses, and protecting the liver from drug-induced injury (Jung *et al.*, 2019).

PC has been used to induce lipolysis in adipose tissues, such as via injection in cosmetic surgery (Jung *et al.*, 2018). The stored triglycerides released from lysed adipocytes are subsequently solubilized and intracellular lipases are activated. A mixture of PC and sodium deoxycholate has been used worldwide for many years as an off-label strategy to reduce the cosmetic volume of subcutaneous fat tissues in the clinic. Currently, only deoxycholate is FDA-approved for this purpose; it is a component of Kybella (Allergan, Irvine, CA, USA), the subcutaneous injection of which is used to improve the appearance of moderate to severe convexity or fullness

**Table 3.** Biomedical activity and application of glycerophospholipids

Subclass	Name	Activity	Medical application	Ref.
PC	PC	Lipolysis in human fat tissue	Cosmetic procedure	Thomas <i>et al.</i> , 2016
		Lipolysis in malar, jawline, submental areas and upper arm	Cosmetic procedure	Thomas <i>et al.</i> , 2018
		Promote adipocyte-specific lipolysis and apoptosis through TNF- $\alpha$ or IL-1 $\beta$ signaling	Adipocyte lipolysis and apoptosis	Jung <i>et al.</i> , 2019
		Reduce gastric bleeding, prevent GI permeability disturbance due to LPS	Gastrointestinal tract inflammation	Dial <i>et al.</i> , 2008
		Improves intestinal barrier function.	Drug-induced Liver injury	Chen <i>et al.</i> , 2019
	Polyenylphosphatidylcholine	Protective effects against lipid peroxidation, oxidative stress and hepatic fibrosis	Fatty liver, metabolic comorbidities	Maev <i>et al.</i> , 2020
PI	Phosphatidylinositol (3, 4, 5) – trisphosphate (PI(3,4,5)P3)	Signaling molecule processed by phosphatidylinositol 3-kinase	Insulin resistance	Kachko <i>et al.</i> , 2015
PS	PS	Suppress the TNF- $\alpha$ production of peritoneal macrophage	Inflammatory disease	Klein <i>et al.</i> , 2020
		Interfere with the pro-inflammatory cytokine formation of phagocyte	Inflammatory disease	Szondy <i>et al.</i> , 2017
	Lecithin-transphosphatidylated PS	Restored age-associated decreases of choline transporter	Memory impairment	Lee <i>et al.</i> , 2015
Plasmalogen	Ethanolamine plasmalogen	Correlation of declined circulating plasmalogen and cognitive function	Alzheimer’s disease	Wood <i>et al.</i> , 2010
		Enhanced inhibition activity of phosphatidylethanolamine on $\gamma$ -secretase	Alzheimer’s disease	Onodera <i>et al.</i> , 2015
		Improvement of cognitive functions	Alzheimer’s disease	Fujino <i>et al.</i> , 2017
		Improved memory impairment in amyloid $\beta$ -infused rats	Alzheimer’s disease	Yamashita <i>et al.</i> , 2017



associated with submental fat in adults.

Although not yet approved by FDA, PC has been proven to be effective in lipolysis in animals and humans when combined with deoxycholate. It has been shown to promote lipolysis effects with fewer side effects than deoxycholate alone (Salti *et al.*, 2008), and local application of a PC/deoxycholate cocktail has been repeatedly shown to reduce unwanted fat with improved effects at the face (malar, jawline, and submental areas) and upper arm and average effects at the thighs and knees (Thomas *et al.*, 2016, 2018).

PC treatment was also reported to improve intestinal barrier functions. PC is an important surface-active compound that generates the hydrophobic biophysical surface property of the intestine, thereby protecting against microorganisms and toxic byproducts. Orally administered PC (100 mg/kg) was found to protect the intestinal mucosa from LPS-induced damage and potentially prevent LPS-induced gastric bleeding (Dial *et al.*, 2008). The study revealed that PC acted directly in the mucosa to prevent LPS from disturbing the permeability of the gastrointestinal tract.

Patients with liver cirrhosis or hepatocarcinoma show considerable increases of lysophosphatidylcholine in the fecal, which indicates the reduction of PC in the intestinal lumina (Huang *et al.*, 2013). A recent study reported that oral PC improved liver function following CCl<sub>4</sub>-induced injury (Chen *et al.*, 2019). Oral PC could be directly absorbed into the mucus layer, thereby restores the intestinal barrier function, triggers reduction of the portal pressure and improves the liver function. In clinical trials, polyenylphosphatidylcholine, an unsaturated fatty acid PC, is used as an adjunctive therapy in improving the liver function of patients with nonalcoholic fatty liver disease and metabolic comorbidities (Maev *et al.*, 2020). The protective effect of polyenylphosphatidylcholine against nonalcoholic fatty liver disease suggests its function in suppressing lipid peroxidation, oxidative stress, and hepatic fibrosis.

### Phosphatidylinositol (PI)

PI is a low-abundance phospholipid that constitutes 5-8% of the total phospholipid component of the cell membrane. It shares some structural similarity with the major phospholipids, but is distinguished by the presence of an inositol sugar moiety at the polar head. The mammalian PI derivatives tend to have stearic acid (C18:0) or arachidonic acid (C20:4) as the fatty acid chains linked to their glycerol backbone. Among them, phosphatidylinositol-4,5-diphosphate (PI(4,5)P<sub>2</sub>) is the most abundant phosphoinositide, accounting for 1-2% of total lipids. It regulates various aspects of cellular function, including cytoskeletal organization, membrane trafficking, and ion channels (Schink *et al.*, 2016). PI(3,4,5)P<sub>3</sub>, which is mainly generated by phosphorylation of PI(4,5)P<sub>2</sub> via phosphatidylinositol 3-kinase, plays important roles as a signal mediator for various cellular functions, such as cell motility, cell metabolism, cell survival, and gene regulation (Czech, 2003; Marat and Haucke, 2016).

PI products have also been shown to trigger downstream signals and induce translocation of the insulin-sensitive glucose transporter, GLUT4, from internal membrane pools to the plasma membrane in muscle and fat cells (Draznin, 2006; Bridges and Saltiel, 2015). Therefore, PI(3,4,5)P<sub>3</sub> could be helpful in relieving insulin resistance. Indeed, intracellular delivery of PI(3,4,5)P<sub>3</sub> using polymeric nanoparticles was shown to overcome insulin resistance. The researchers condensed

polyethyleneimine (PEI) 25 kDa and PI(3,4,5)P<sub>3</sub> into PEI-25/PIP<sub>3</sub> nanoparticles whose cationic charge (derived from PEI<sub>25</sub>) caused PI(3,4,5)P<sub>3</sub> to be retained at the cell perimeter. This strategy was thought to mimic the extent and cellular organization of PI(3,4,5)P<sub>3</sub>, which is preferable for insulin stimulation (Kachko *et al.*, 2015).

### Phosphatidylserine (PS)

PS is an anionic phospholipid found abundantly in the inner leaflet of eukaryotic membranes. Structurally, PS consists of a glycerol backbone, two fatty acid chains of variable length and saturation, and a phosphate group conjugated with a polar serine moiety. When cells undergo apoptosis, the asymmetric distribution of PS across the lipid bilayer is disrupted by the scramblase enzyme, which is activated during apoptosis. PS is exposed in the outer leaflet, where it is recognized by phagocytes as an “eat me” signal.

Phagocytosis of apoptotic cells is triggered by the interaction of PS with PS-specific receptors, such as stabilin-2, brain-specific angiogenesis inhibitor 1 (BAI1), and members of the T-cell immunoglobulin and mucin (TIM) family. The phagocytosis of PS-exposing cells is also mediated by bridging molecules, such as milk fat globulin-EGF-factor 8 and growth arrest-specific gene 6, which bind to PS-exposing cells and bridge the interaction of phagocytes and apoptotic cells. Both interaction pathways generate an “eat me” signal to phagocytes, resulting in the clearance and removal of apoptotic cells. This particular bioactivity of PS has been recognized as a major feature of immunotherapy and has been applied in recent studies.

In order to promote drug delivery into activated microglia, researchers prepared PS-microbubbles by mixing PS with 2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (DSPE-PEG2000), and 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (Zhao *et al.*, 2018). The PS-decorated microbubbles were specifically taken up by microglia in inflammatory areas of a model of cerebral ischemia. The uptake of PS-microbubbles by macrophages was double that seen for control-microbubbles. Following intravenous administration of PS-microbubbles, ultrasound was used to decompose the particles from micro- to nano-sized, to facilitate penetration of the blood-brain barrier. In a similar study, acetylated dextran nanoparticles were coated with 1, 2-dipalmitoyl-sn-glycero-3-phospho-L-serine to promote the uptake of curcumin by macrophages (Shah *et al.*, 2019). PS-coated dextran nanoparticles were taken up at a 4-fold higher rate by RAW 264.7 macrophages compared to control A549 lung cancer cells.

PS delivered in a micelle formulation was recently shown to stimulate the polarization of macrophages from the pro-inflammatory to anti-inflammatory phenotype (Klein *et al.*, 2020). This was consistent with previous reports associating PS treatment with down-regulation of pro-inflammatory cytokines (e.g., TNF- $\alpha$  and IL-6) and increased secretion of anti-inflammatory mediators (e.g., IL-10 and TGF- $\beta$ ) (Szondy *et al.*, 2017). Indeed, micelles enriched with 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS) significantly reduced the release of TNF- $\alpha$  by LPS-activated macrophages, indicating an anti-inflammatory effect (Klein *et al.*, 2020).

Oral administration of PS was found to improve memory impairment in aged rats (Lee *et al.*, 2015). A dose of 20 mg/kg PS was associated with recovery of age-related decreases in the

mRNA levels of choline transporter and muscarinic acetylcholine receptor type 1, and a dose of 50 mg/kg yielded significant memory improvements. This suggests that PS may improve age-related memory deficits and thus could hold promise for use in other diseases associated with neurodegeneration.

### Plasmalogen

Plasmalogen is a major component of the membrane phospholipids found in the brain, kidneys, lungs, and skeletal muscles; it accounts for 18-20% of all phospholipids (Braverman and Moser, 2012). Structurally, plasmalogen consists of a glycerol backbone, a vinyl ether linkage at sn1, a fatty acid ester linkage at sn2, and a phosphate group at sn3 that is tethered with either ethanol amine (to yield ethanolamine plasmalogen) or choline (to yield choline plasmalogen). Ethanolamine plasmalogen is widely distributed in the brain, while choline plasmalogen is found more in heart muscle. Plasmalogen has attracted recent interest because it appears to play important roles in various diseases, such as Alzheimer's disease, cardiovascular disease, cancer, and respiratory disease (Braverman and Moser, 2012; Sutter *et al.*, 2016; Messias *et al.*, 2018).

Increasing evidence suggests that ethanolamine plasmalogen is deficient in Alzheimer's disease: in Alzheimer's disease patients, the ethanolamine plasmalogen level was found to be reduced in post-mortem brain samples as well as in cerebrospinal fluid, plasma, serum, and red blood cells. Compared to healthy brain tissues, ethanolamine plasmalogen was reduced by 70% in the brains of Alzheimer's patients (Wood *et al.*, 2010; Onodera *et al.*, 2015). It is unclear whether ethanolamine plasmalogen deficiency is a cause or a result of Alzheimer's disease, but studies have shown that increasing plasma levels of ethanolamine plasmalogen can improve disease symptoms (Fujino *et al.*, 2017; Yamashita *et al.*, 2017). In a study on amyloid  $\beta$ -infused rats, oral administration of ethanolamine plasmalogen at 209  $\mu\text{mol/kg}$  body weight/day for 4 weeks improved the working memory-related learning ability in tested animals (Yamashita *et al.*, 2017). In a recent human trial, oral administration of ethanolamine plasmalogen at a dose of 1 mg/day for 24 weeks significantly improved memory among female patients with Alzheimer's disease and those under the age of 77 (Fujino *et al.*, 2017).

## SPHINGOLIPIDS

Sphingolipids, which are a main membrane lipid, have one polar head and two aliphatic chains, and play important roles in cell recognition and signaling (Hannun and Obeid, 2008). Sphingolipids are composed of a sphingoid base having an amino alcohol group; other components are connected to a sphingoid base through amide, glycoside, or phosphodiester bonds (Hannun and Obeid, 2018).

The addition of head groups to ceramide produces different types of sphingolipids, such as sphingomyelins, cerebroside, and gangliosides (Hannun and Obeid, 2018). Sphingomyelins consist of a ceramide backbone and phosphocholine or phosphoethanolamine, and thus resemble phospholipids. They are also an important component of the cell membrane, especially that of the myelin sheath that covers neuronal axons (Slotte, 2013). The 1-hydroxyl group of ceramides can be conjugated with carbohydrate residues by  $\beta$ -glycoside linkage to gener-

ate glycosphingolipids, such as cerebroside, globoside, and ganglioside (Zhang *et al.*, 2019a).

Cerebroside has a ceramide backbone with a single sugar, such as glucose or galactose, and are commonly called glucocerebroside (glucosylceramide) or galactocerebroside (galactosylceramide), respectively. Generally, galactocerebroside is abundant in neural tissue, while glucocerebroside is found in other tissues. Ganglioside is a glycosphingolipid with a ceramide backbone with an oligosaccharide that must contain at least one sialic acid residue; this provides a negative charge at physiological pH that distinguishes gangliosides from cerebroside and globoside, which have neutral charges (Cuttillo *et al.*, 2020). Gangliosides are present in the outer membrane of cells and are primarily responsible for recognizing signaling molecules (Cuttillo *et al.*, 2020).

Sphingolipids have been reported to have multiple biological functions. In cellular level, they are involved in providing the structural integrity of cell membrane, cell recognition, cell adhesion, endoplasmic reticulum stress, and controlling the function of cilia and microvilli (Park and Park, 2020). In central nervous system, sphingolipids are known to be players of brain development (Dasgupta and Ray, 2017). In gastrointestinal tract, sphingolipids play a role in regulating oral absorption of some nutrients (Kurek *et al.*, 2013). In immune systems, sphingolipids have been reported to function in the differentiation, maturation, and immune responses of lymphocytes including B cells, and T cells (Zhang *et al.*, 2020).

Due to the various biological functions, sphingolipids and their derivatives have been studied for therapeutic applications of diverse diseases such as cancer, and autoimmune diseases (Table 4). In cancer immunotherapy, targeting specific types of glycosphingolipids has been shown to modulate the immune microenvironments of tumors (Yu *et al.*, 2020). Safingol and fingolimod are examples of sphingolipid analogues for clinical applications (Choi and Song, 2020). Safingol can exert anticancer effect by suppressing the activity of sphingosine kinases. Fingolimod is clinically used for treatment of multiple sclerosis. Fingolimod is an analogue of the sphingolipids that modulates the sphingosine-1-phosphate receptor 1. Phosphorylated fingolimod is reported to internalize the receptors and block the migration of lymphocytes to the central nervous system (Brinkmann *et al.*, 2010).

### Ceramides

A ceramide is composed of a sphingoid base and a fatty acid (Cha *et al.*, 2016). Ceramides can be synthesized from L-serine and palmitoyl-CoA via the *de novo* pathway, from sphingomyelins via the sphingomyelinase pathway, and from complex sphingolipids via the salvage pathway (Simon *et al.*, 2021). Generally, ceramides are associated with cell death, differentiation, proliferation, and inflammation (Turpin-Nolan and Brünig, 2020; Zhang *et al.*, 2020).

Lipid-based nano-carrier systems, such as liposomes, are one of the promising candidates that have been studied for their potential to improve the physicochemical limitations of ceramides. For example, C6 ceramide encapsulated liposomes are known to induce caspase-mediated apoptosis more potently in osteosarcoma cells than free C6 ceramide, thereby providing better safety against non-cancerous bone cells. Moreover, administration of liposomal C6 ceramide with methotrexate had synergistic effects compared to monother-

**Table 4.** Biomedical activity and application of sphingolipids

Subclass	Name	Activity	Medical application	Ref.
Ceramide	C6 ceramide	Increasing vincristine sensitivity via AMP activated protein kinase–p53 signaling	Colon cancer, pancreatic cancer	Chen <i>et al.</i> , 2015
		Oxidized graphene nanoparticles as a delivery system for the pro-apoptotic C6 ceramide	Cervical cancer	Suhrland <i>et al.</i> , 2019
		Graphene oxide nanoparticle delivery system enhance anti-cancer activity of C6 ceramide	Hepatocellular carcinoma	Wang <i>et al.</i> , 2019
		Liposomal short-chain C6 ceramide induces potent anti-osteosarcoma activity	Osteosarcoma	Zhai <i>et al.</i> , 2015
Sphingo-myelin	Sphingomyelin	Sphingomyelin-based nanosystems (SNs) for the anticancer miRNA therapeutics	Colon cancer	Nagachinta <i>et al.</i> , 2020
		Site-activated drug releasing lipid-iron nanoparticle	Cancer	Medina <i>et al.</i> , 2020
		Engineered liposomes sequester bacterial exotoxins and protect from invasive infections	Antibiotics	Henry <i>et al.</i> , 2015
		Specific drug release lipid nanoparticles at infection sites	Antibiotics	Zhang <i>et al.</i> , 2021
Glyco-sphingolipid	N-octanoyl-glucosylceramide	Improvement of doxorubicin delivery and efficacy in solid tumors	Squamous cell carcinoma, Melanoma	van Lummel <i>et al.</i> , 2011
		Improvement of efficacy of liposomal doxorubicin	Lobular breast carcinoma	van Hell <i>et al.</i> , 2013
		Formation of transient micro-channels within the cell membrane	Uterine sarcoma	Zalba <i>et al.</i> , 2020
	$\alpha$ -Galactosyl-ceramide ( $\alpha$ -GalCer)	Antigen-presenting cell immunotherapy by the activation of endogenous iNKT cells	Non-small cell lung cancer	Toyoda <i>et al.</i> , 2020
		mRNA nanovaccine with $\alpha$ -GalCer induces antitumor immunity by NKT cells	Melanoma	Verbeke <i>et al.</i> , 2019

apy (Zhai *et al.*, 2015). However, although liposome formulations can provide improved physical properties and efficacy for C6 ceramide, several drawbacks, such as poor loading efficiency due to other lipids and cofactors, limit their use (Khanzhanov *et al.*, 2008).

Graphene nanosheets can also be used to deliver lipids attached via hydrophobic interactions (Shim *et al.*, 2016). Graphene nanosheets and oxidized graphene nanoribbons have been studied for the delivery of C6 ceramide. Mass spectrometry showed that the loading efficiencies of C6 ceramide onto the graphene nanosheets and oxidized graphene nanoribbons were 51.5% and 57%, respectively, and these formulations were found to reduce the viability of HeLa cells through caspase-3 dependent apoptosis (Suhrland *et al.*, 2019). Additionally, modified C6 ceramide-loaded graphene nanosheets showed promising anti-cancer effects in a hepatocellular carcinoma model. The graphene oxide nanosheets were conjugated with polyethyleneglycol and PEI to enhance cellular uptake via a clathrin-mediated mechanism. The surface-modified nanosheets inhibited tumor growth and improved the survival duration in a hepatocellular carcinoma xenografts mouse model, and exhibited synergistic effects with sorafenib (Wang *et al.*, 2019).

### Sphingomyelin

Sphingomyelin is a major structural lipid that contributes to forming the plasma membranes of mammalian cells (van

Meer *et al.*, 2008). Sphingomyelin has phosphocholine or phosphoethanolamine and thus shares physicochemical properties with phospholipids such as PC (Slotte, 2013). Similar to PC and other phospholipids, sphingomyelin provides high bioavailability for liposomal drug delivery systems. A sphingomyelin-based nanosystem was recently investigated for miRNA delivery (Nagachinta *et al.*, 2020). The lipid formulations comprised vitamin E, stearylamine or N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP), PEG<sub>12</sub>-C<sub>18</sub>, and sphingomyelin.

Two strategies for loading miRNA145 were investigated: attaching the miRNA to the surface of a lipid nanoparticle composed of stearylamine and sphingomyelin; and encapsulating a lipid complex composed of miRNA and DOTAP inside the sphingomyelin lipid layer (SNs-Lpx). The generated sphingomyelin-based miRNA-encapsulated lipid nanoparticles showed higher cellular uptake, transfection efficiency, and anticancer effects compared to miRNA-tethered liposomes composed of stearylamine and sphingomyelin. This likely reflects that the encapsulation of miRNA inside the sphingomyelin lipid layer avoided its premature release (Nagachinta *et al.*, 2020).

Sphingomyelin has also been included in environmentally reactive drug delivery systems developed using enzymes that are induced by specific environmental cues. The members of the acid sphingomyelinase family of sphingomyelinases are activated by oxidative stress or ionizing radiation (Paris *et al.*, 2001) and function to convert sphingomyelins to ceramide

(Ogretmen, 2017). Iron nanoparticle-containing sphingomyelin lipid nanoparticles have been developed for site-specific drug release (Medina *et al.*, 2020). In this study, the lipid nanoparticles were composed of DSPC, DOTAP, cholesterol, and sphingomyelin. The particles were designed to be broken down by acid sphingomyelinase-secretory cells, such as those represented by the cisplatin-exposed SCC-9 cell line (Lacour *et al.*, 2004), and could be remotely localized to the tumor site by altering magnetic field (AMF), due to the presence of iron nanoparticles in the lipid layer. In the orthotropic SCC-9 tumor bearing mouse model, lipid-iron nanoparticles were specifically delivered to tumor tissue, where they released indocyanine green and cisplatin for diagnostic and therapeutic purposes, respectively (Medina *et al.*, 2020).

Pore-forming toxins originating from bacteria have the ability to form membrane pores in the host cell membrane (Parker and Feil, 2005). Cholesterol-dependent cytolysis involving the actions of the pore-forming toxins such as pneumolysin, streptolysin O, tetanolysin, and  $\alpha$ -hemolysin is important for the progression of infectious diseases. Thus, cholesterol and sphingomyelin-based liposomes are being investigated as a means to attenuate these toxins by mimicking the mammalian cell membrane (Henry *et al.*, 2015). Cholesterol-rich sphingomyelin liposomes were generated and found to competitively prevent the binding of pneumolysin,  $\alpha$ -hemolysin, and phospholipase C. In other work, liposomes were used to protect mice from sepsis caused by *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*) and prevent pneumococcal pneumonia (Henry *et al.*, 2015).

Pore-forming toxins also recognize certain lipids on the surface of the host membrane; these include sphingomyelin, which is well represented on animal cell membranes (Parker and Feil, 2005). As a result, sphingomyelin liposomes that respond to pore-forming toxins and release encapsulated antibiotics were developed as a means to combat infectious diseases. These liposomes comprised unsaturated fatty acid-bound phospholipids that provide fluidity to the membrane, and encapsulated cholesterol, sphingomyelin, and vancomycin inside the hydrophilic core. The liposomal vancomycin was released in the  $\alpha$ -hemolysin rich environment, and its antibacterial efficacy was demonstrated in both *in vitro* and *in vivo* models (Zhang *et al.*, 2021).

### Glycosphingolipids

Cerebroside is a ceramide that binds to a single sugar molecule, usually glucose or galactose (Zhang *et al.*, 2019a). Glucosylceramides (or glucosylcerebrosides), especially those conjugated with a short-chain fatty acid, have been studied to control cell plasma membrane fluidity. N-octanoyl-glucosylceramide was found to cause incomplete lipid packing and enhance the cellular uptake of amphiphilic drugs, such as doxorubicin (van Lummel *et al.*, 2011). This short-chain glucosylceramide generated transient membrane channels to mediate the translocation of doxorubicin, and thus improving its efficacy (van Hell *et al.*, 2013). This strategy may be a promising solution for multi-drug resistant (MDR) cancer therapy. Doxorubicin-resistant cancer cells have relatively rigid lipid membranes compared to those of doxorubicin-sensitive cancer cells, and this rigidity can interfere with drug absorption. As a result, the use of N-octanoyl-glucosylceramide modified liposomes loaded with doxorubicin yielded improved doxorubicin accumulation in tumor tissues compared to a conventional

liposome formulation (Zalba *et al.*, 2020).

Glycosphingolipids, including cerebroside, play important roles in the immune system. Immune cells have their own distinct glycosphingolipid expression patterns in the cell membrane, and these patterns are associated with cell proliferation and/or differentiation. For example, glycosphingolipids are essential components of glycosphingolipid-enriched microdomains (GEMs) and control cell activity (Zhang *et al.*, 2019a).  $\alpha$ -Galactosylceramide ( $\alpha$ -GalCer), which is composed of  $\alpha$ -linked galactose and ceramide, is a lipid antigen for invariant natural killer T cells (iNKT cells).  $\alpha$ -GalCer is recognized by the invariant T cell receptor (invTCR) of iNKT cells via the MHC class I-like molecule, CD1d; this triggers the iNKT cells to release Th1 and Th2 cytokines, which activate leukocytes, such as dendritic cells and T cells (Zhang *et al.*, 2019b). Therefore,  $\alpha$ -GalCer has been investigated for cancer immunotherapy through iNKT cell activation (Toyoda *et al.*, 2020). In a study on an mRNA vaccine nanoparticle formulation,  $\alpha$ -GalCer was used to target NKT cells and DOTAP provided cationic properties for mRNA loading. The  $\alpha$ -GalCer-based nanoparticles were found to activate both innate and adaptive immunity and exhibit improved anticancer efficacy (Verbeke *et al.*, 2019).

The lack of satisfactory results in clinical studies using  $\alpha$ -GalCer alone underlined the need to develop stronger immune-activating reagents, prompting researchers to develop  $\alpha$ -GalCer analogs for cancer immunotherapy and test vaccine adjuvants, such as 7DW8-5 (Li *et al.*, 2010). Indeed, 7DW8-5-encapsulating liposome therapy showed synergistic effects with CAR-T cell therapy in solid tumors. As a glycolipid adjuvant, 7DW8-5 was entrapped inside the liposome with a PI3K inhibitor intended to recover the immunosuppressive tumor microenvironment, and the particle was modified with the tumor-targeting peptide, iRGD (Zhang *et al.*, 2018). 7DW8-5 has also been applied to manufacture influenza vaccines and strengthen the production of influenza virus-specific antibodies in mouse models (Feng *et al.*, 2019).

## SACCHAROLIPIDS

Saccharolipids have a structure in which fatty acids are linked to the sugar backbone. The different types of saccharolipids are classified by the different structures formed by the sugar moiety, and there are two main subclasses: acylaminosugars and acyltrehaloses. Examples of biomedical applications of saccharolipids are listed in Table 5.

### Lipopolysaccharide (LPS)

LPS consists of a lipid and O-antigen-containing polysaccharide. It is also known as endotoxin, as it is a toxin located within bacterial cells. Lipid A, which is an acylaminosugar present in gram-negative bacteria, is a component of LPS, along with O-antigen and oligosaccharides. The structure of LPS allows bacteria to maintain the integrity of the cell wall, to which it is tethered by lipid A. When the cell wall is decomposed, lipid A is released from the bacterial wall to exert a toxic effect on the human body. The complement cascade is initiated by C3a and C5a in macrophages, which causes the release of histamine and triggers an inflammatory response via the release of TNF, cytokines, interleukins, and prostaglandins. Topical administration of bacterial LPS was used to treat

**Table 5.** Biomedical activity and application of saccharolipids

Subclass	Name	Activity	Medical application	Ref.
Lipid A	LPS	Upregulation of pro-inflammatory cytokines	Wound healing	Kostarnoy <i>et al.</i> , 2013
		Generate tolerogenic dendritic cells to exhaust effector CD4 T cells	Experimental autoimmune encephalomyelitis	Zhou <i>et al.</i> , 2014
		Increase endogenous TNF- $\alpha$ expression	Cancer	Goto <i>et al.</i> , 1996
		Upregulation of MHC molecule on GSCs	Cancer	Han <i>et al.</i> , 2017
Monophosphoryl lipid A	TLR4 agonist	Enhancing CD4 T cell activation and initial clonal expansion	Vaccine adjuvant	Saito <i>et al.</i> , 2010
		Enhancing CD4 T cell activation and initial clonal expansion	Vaccine adjuvant	Thompson <i>et al.</i> , 2005

skin wounds in mice. It promoted inflammation resolution and macrophage infiltration, thereby enhancing collagen synthesis. Administration at the wound site increased the levels of the CC chemokines (CCL2/monocyte chemoattractant protein-1, CCL7/MCP-3, CCL3/MIP-1 $\alpha$ , and CCL5/RANTES), which are associated with macrophage infiltration, and increased the collagen synthesis-related cytokines, TGF- $\beta$ 1 and fibroblast growth factor 2 (FGF2) (Kostarnoy *et al.*, 2013).

LPS treatment generated apoptotic dendritic cells, which triggered the generation of tolerant dendritic cells to deplete effector CD4<sup>+</sup> T cells in an autoimmune encephalomyelitis murine model, supporting the potential of LPS-induced dendritic cell therapy for multiple sclerosis (Zhou *et al.*, 2014). There is controversy over the use of LPS in antitumor cancer therapy. LPS treatment was reported to confer antitumor effects by upregulating endogenous TNF, and LPS combined with cyclophosphamide synergistically increased the antitumor effect of TNF (Goto *et al.*, 1996). LPS-pretreated glioma stem-like cells (GSCs) showed upregulation of MHC molecules, and LPS-pretreated GSC-inoculated rats had a longer mean survival time compared to those treated with control GSC (67.0  $\pm$  31.8 days vs. 16.0  $\pm$  2.5 days, respectively) (Han *et al.*, 2017). A high dose of LPS in colorectal cancer was found to interfere with the effect of anti-programmed death-ligand 1 monoclonal antibody (anti-PD-L1 mAb) treatment. However, LPS-targeting fusion protein-loaded nanoparticles were found to improve colorectal cancer immunotherapy outcomes (Song *et al.*, 2018).

LPS, which is derived from Gram-negative bacteria, is often heterogeneous. Differences in the lengths and compositions of the terminal glycan chains may lead to different experimental consequences, such as in the context of Toll-like receptor 4 (TLR4). To address this issue, researchers developed di[3-deoxy-D-manno-octulosonyl]-lipid A (Kdo<sub>2</sub>-Lipid A), which is a homologous subclass of LPS that exhibits endotoxin activity similar to LPS and is highly selective for TLR4. Kdo<sub>2</sub>-Lipid A outperforms LPS in yielding highly reproducible results with 10-fold higher endotoxin activity (Saito *et al.*, 2010).

Several limitations have been proposed for LPS-based immunotherapy, including the development of LPS resistance, the existence of sensitivity differences between *in vivo* mouse models and humans, and the risk of adverse effects such as systemic inflammatory response syndrome, generalized Schwartzman reaction, hepatotoxicity, and Arthus reaction (Shetab Boushehri and Lamprecht, 2019). Various efforts have been made to overcome these issues, such as developing lipid A moieties to reduce the risk of side effects or resistance. Among them, tolerin and SDZ MRL953 could

prevent sepsis, ONO-4007 showed tumor regression, and monophosphoryl lipid A was used as cancer vaccine adjuvant (Rockwell *et al.*, 2010). Other efforts include the development of controlled drug-delivery strategies, such as by engineering sustained release of LPS from nanoparticles, the use of microneedles for passive targeting of antigen-presenting cells, and targeting of intracellular LPS to downregulate unwanted pro-inflammatory pathways (Shetab Boushehri *et al.*, 2019).

### Monophosphoryl lipid A

LPS has been excluded from clinical trials due to its high toxicity, but monophosphoryl lipids exhibit far less toxicity. Monophosphoryl lipid A is a low-toxicity derivative of LPS that can be used as a TLR4 agonist and to promote CD4<sup>+</sup> T cell activation. In an early study, monophosphoryl lipid A was emulsified in water with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and administered intravenously, and this formulation triggered CD4<sup>+</sup> T cell activation and initial clonal expansion comparable to that obtained with LPS, but with lower toxicity (Thompson *et al.*, 2005). Since then, monophosphoryl lipid A has been approved as a vaccine adjuvant in liposome formulations (Bashiri *et al.*, 2020).

## CURRENT CHALLENGES AND PERSPECTIVES

The successful use of lipids in preventing and treating chronic diseases has been increasingly demonstrated in recent studies. Their therapeutic effect is seen both as monotherapy and as an adjuvant in combination with other drugs or substances (MacLeod *et al.*, 2011). Due to the special physicochemical properties of lipids, including their low water solubility, high oil-water partition coefficient, high melting point, and crystal state at room temperature, therapeutic lipids face issues with their formulation, dosing, absorption and stability (McClements, 2010). Moreover, lipids are susceptible to oxidation over time, which can reduce their therapeutic activity and/or produce toxic by-products (Khanum and Thevanayagam, 2017). To overcome these problems, several strategies have been studied to extend the application and efficacy of bioactive lipids, as described in the following.

Fatty acids are mostly administered by the oral route (N'Goma *et al.*, 2012). Because fatty acids are not freely dispersed in the aqueous phase, they tend to form large fat globules in the gastrointestinal tract. The enzymatic de-esterification of these lipids occurs at the water-oil interface. Therefore, the smaller the lipid droplets, the faster they hydrolyze and higher the bioavailability (Maki and Dicklin, 2019). Of note,

the absorption of these lipids is highly dependent on dietary fat, which activates the release of bile acids to emulsify the consumed lipids. Therefore, high intra-individual variation in bioavailability has been a challenge in clinical trials (Bisschop *et al.*, 2004). Efforts have been made to reduce the size of lipid droplets in the gastrointestinal tract using surfactants or emulsifiers. For example, omega-3 ethyl ester exhibited improved bioavailability when combined with a non-ionic surfactant mixture consisting of polysorbate 80 and polyoxyethylene-polyoxypropylene block copolymer (Lopez-Toledano *et al.*, 2019).

Another problem with lipid formulations is the risk of lipid peroxidation, which occurs by via pathways in bulk lipid materials and emulsification systems. Lipid peroxidation, which is generally caused by free radicals or enzymes (Khanum and Thevanayagam, 2017), generates secondary oxidation products, such as aldehydes, ketones, alcohols, hydrocarbons, organic acids, and epoxy compounds (Vieira *et al.*, 2017). These substances potentially cause oxidative stress and damage to the human body (Ramana *et al.*, 2013). To prevent peroxidation, many strategies have been proposed for the design and preparation of lipid products. During the preparation phase, lipid peroxidation may be reduced by carefully controlling the materials and manufacturing conditions, such as by using high-purity ingredients, monitoring the pH levels during formulation and storage, and reducing or removing trace metals. During the design phase, lipid peroxidation can be reduced by including antioxidants, such as ascorbic acid,  $\alpha$ -tocopherol, propyl gallate, or ascorbyl palmitate, in the aqueous phase (Khanum and Thevanayagam, 2017).

Many lipids have been approved for human use in both clinical trials and commercial products. They come in a variety of pharmaceutical forms or supplement products. In the context of drug delivery systems, lipids are used in a variety of nano- and micro-formulations, such as liposomes, micelles, and solid lipid nanoparticles. In addition to natural lipids, synthetic lipids are also used in specific applications, such as gene transfer. mRNA vaccines are being increasingly investigated in lipid-based formulations, due to the many reports indicating the safety and efficacy of lipid-based delivery systems. Several lipid nanoparticle-based SARS-CoV-2 mRNA vaccines have been approved for human use. For example, lipid nanoparticles made of the proprietary ionic lipid SM-102, polyethyleneglycol 2000, dimyristoyl glycerol, cholesterol, and DSPC were developed by Moderna (Cambridge, MA, USA) and achieved a vaccine effect of 94.5% against SARS-CoV-2. BioNTech SE (Mainz, Germany) developed another effective lipid nanoparticle candidate. Formulated with the lipids, ((4-hydroxybutyl)azanediyl) bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 2 [(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-distearoyl-sn-glycero-3-phosphocholine, and cholesterol, it showed promising protective effects (Milane and Amiji, 2021). Additional lipid nanoparticle formulations are currently being developed for mRNA vaccine delivery in other diseases, such as cancer and infectious diseases. (Miao *et al.*, 2019; Zhang *et al.*, 2019c).

## CONCLUSION

New lipid species and their biological functions in pathology are increasingly being elucidated. Lipids show potential as new drug candidates and components of lipid-based nanomedicines.

With the increased interest in lipid-based nanomaterials for delivering genes and vaccines, the study of functional lipids will continue to grow. The potential therapeutic applications of lipids have been supported in various diseases, emphasizing the importance of lipids in biomedicine. Although issues with the physical stability and formulation needs of lipid-based drugs still remain as challenges, continued advances in basic science are expected to facilitate the discovery of new species and roles of lipid in the future, further extending the application of lipids in efforts to treat and/or prevent various diseases.

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