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The role of sphingolipids in endothelial barrier function

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Abstract

Sphingolipids are a ubiquitous family of essential lipids with an increasingly understood role as biologically active mediators in numerous physiologic and pathologic processes. Two particular sphingolipid species, sphingosine-1-phosphate and ceramide, and their metabolites interact both directly and indirectly with endothelial cells to regulate vascular permeability. Sphingosine-1-phosphate generally augments endothelial integrity while ceramide tends to promote vascular leak, and a tight balance between the two is necessary to maintain normal physiologic function. The mechanisms by which sphingolipids regulate endothelial barrier function are complex and occur through multiple different pathways, and disruptions or imbalances in these pathways have been implicated in a number of specific disease processes. With improved understanding of sphingolipid biology, endothelial function, and the interactions between the two, several targets for therapeutic intervention have emerged and there is immense potential for further advancement in this field.

Keywords

ceramide; endothelial barrier; endothelial cells; endothelium; sphingolipids; sphingosine-1-phosphate

Introduction

Sphingolipids and the vascular endothelium have traveled oddly similar paths since their initial discoveries. Sphingosine was described in the 1880s, around the same time a cellular layer was discovered lining the inside of blood vessels (Thudichum, 1884; Cines et al., 1998). For roughly 60 years, both were viewed as largely structural and physiologically inert and there was a lack of reliable methods with which to study them. Only over the past few decades have the true nature and activity of sphingolipids and endothelial cells begun to be recognized. The introduction of new techniques and models such as inhibitors of sphingolipid metabolism, immortalized endothelial cell lines, and knockout mice opened the door for new developments in both fields. Since then, sphingolipids and endothelial cells have emerged as key players in countless physiologic and pathologic processes with significant overlap between the two. There is mounting evidence of important interactions between sphingolipids and the endothelium relating to angiogenesis, vascular permeability,

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immune cell migration, and inflammatory pathways. In this review, we will explore the existing literature on the relationships between sphingolipids and endothelial barrier function as well as implications in specific pathological entities and opportunities for therapeutic intervention.

Sphingolipid structure and metabolism

The term ‘sphingolipids’ encompasses a large, heterogeneous family of essential lipids that are characterized by a sphingoid backbone that is N-acylated with fatty acids. Modifications, such as addition of different head and tail groups, can lead to generation of hundreds of different subspecies, each conferred with unique structure and bioactivity (Hannun and Obeid, 2008). An in-depth review of sphingolipid metabolism is beyond the scope of this review, and excellent comprehensive reviews on this subject have recently been published (Gault et al., 2010; Tidhar and Futerman, 2013). However, a brief overview of the key pathways and metabolites is warranted. Central to all sphingolipid pathways is ceramide. Ceramide (actually a family of structurally similar sphingolipids with variable-length fatty acid tails and unique biological properties) can be synthesized *de novo*, released by hydrolysis of sphingomyelin, or recycled through salvage pathways. In the *de novo* pathway, condensation of serine and palmitoyl CoA by serine palmitoyltransferase occurs in the endoplasmic reticulum and forms 3-keto-dihydrosphingosine, which is then reduced to dihydrosphingosine (Linn et al., 2001; Hannun and Obeid, 2002). Addition of a fatty acid 14–26 carbons long is carried out by one of six ceramide synthases (CerS1-CerS6), followed by dehydration to ceramide (Maceyka and Spiegel, 2014). Formation of ceramide from sphingomyelin occurs by the action of one of five known sphingomyelinases: the lysosomal acid sphingomyelinase (ASM), secretory ASM, magnesium-dependent neutral sphingomyelinase, magnesium-independent neutral sphingomyelinase, or alkaline sphingomyelinase (Uhlig and Gulbins, 2008). Ceramide can undergo phosphorylation by ceramide kinase to form ceramide-1-phosphate, glycosylation to form complex glycosphingolipids, or addition of a phosphocholine group by sphingomyelin synthase to form sphingomyelin. Ceramide may also be degraded by one of the ceramidases to form sphingosine, which may subsequently be phosphorylated to sphingosine-1-phosphate (S1P) by sphingosine kinase 1 or 2 (SphK1 or SphK2) (Hannun and Obeid, 2008). S1P can in turn be degraded by S1P lyase, or it may be recycled back into the sphingolipid pathway by one of the S1P phosphatases. Each of these compounds exhibits unique regulatory and signaling properties. The ASM is ubiquitous and is particularly abundant both in a form that is bound to endothelial cell membranes and free-floating in body fluids (Marathe et al., 1998; Takahashi et al., 2000). Likewise, the sphingosine kinases are present throughout the body and translocate to the plasma membrane when activated by one of a myriad of stimuli (Maceyka and Spiegel, 2014). While all of the sphingolipids exhibit unique biological properties, ceramide and S1P are the best characterized. The relative concentrations of these two molecules are carefully balanced throughout the body in a series of interactive pathways known as the sphingolipid rheostat (Spiegel and Milstien, 2003). Both molecules interact extensively with the endothelium where they play an important part in vascular function and certain pathologic states.

Endothelial structure and function

The endothelium is made up of a continuous monolayer of cells that coat the entire vascular tree. Traditionally, the endothelium was viewed as a static semipermeable membrane, but recent discoveries have revealed key roles in diverse homeostatic processes including vasomotor tone, cell and solute trafficking, immune response, vascular permeability, and angiogenesis (Pries and Kuebler, 2006). The complexity of the endothelium is striking, demonstrating heterogeneity of both structure and function between different vessel types, different organs, different regions within the same tissue, and even between different segments of the same local vessel. Structurally, the endothelium may be continuous as in the blood-brain barrier, fenestrated as in the glomerulus of the kidney, or discontinuous with large sinusoids as in the liver or marrow. This leads to broad differences in baseline function throughout the body. Variability in expression of enzymes, structural proteins, and cell surface markers results in endothelial cells with distinct phenotypic subtypes (Cines et al., 1998). Regional factors also contribute heavily to the differentiation process and can result in varied physiological functions and stress responses by endothelial cells in different environments (Uhlig et al., 2014).

Interendothelial junctions (IEJs) form the structural attachment between endothelial cells and regulate the function of the endothelial barrier, both by opening and closing to selectively allow passage of cells and solutes and by transducing intracellular signals. Low molecular weight solutes (<3 nm) pass through unimpeded by passive diffusion, while macromolecules, proteins, or cells undergo more controlled trafficking by transcellular or paracellular pathways (Mehta et al., 2014). There are several types of recognized IEJs, but the two that that are primarily recognized in interendothelial cell adhesion are adherens junctions (AJs) and tight junctions (TJs). Each of these adhesive structures consists of a unique transmembrane protein that links extracellular adhesion molecules to the actin cytoskeleton by way of an intermediary protein complex (Bazzoni and Dejana, 2004). The cadherins [particularly vascular endothelial (VE)-cadherin] complex with catenins, plakoglobin, and p120 to form AJs while the claudins (primarily claudin 5), occludin, and junctional adhesion molecule A (JAM-A) are the primary mediators of TJs. Additional support and regulatory capacity comes from multiple intracellular proteins, including the zona occludens (ZO) proteins, which are primarily found in TJs but may also have a role early in the formation of AJs (Gumbiner, 2005; Furuse and Tsukita, 2006). In contrast to their predictable location and conformation in epithelial cells, AJs and TJs in endothelial cells are variably positioned on the cell membrane. They are sometimes found in close proximity to each other, and there is some evidence that AJs may have a modifying effect on TJs, although the exact nature of their interaction remains to be fully elucidated (Ruffer et al., 2004; Taddei et al., 2008). Beyond their structural role in cell adhesion, these junctions also have complex signaling functions with both direct and indirect roles in vascular homeostasis, immune cell trafficking, cell differentiation, and apoptosis (Dejana, 2004).

Vascular permeability is tightly regulated by the endothelium (Stevens et al., 2000). Intercellular barrier function is primarily regulated by the intercellular adhesions above, while further contributions come from the basement membrane and the luminal coating of proteoglycans, hyaluronan, and glycoproteins known as the gylcocalyx (Qiao et al., 1995;

Weinbaum et al., 2007). Permeability can be induced by a number of mediators such as thrombin, platelet-activating factor (PAF), bradykinin, histamine, VEGF, and a host of proinflammatory cytokines, while S1P and angiopoetin-1 preserve and augment barrier function (Mehta and Malik, 2006). VE-cadherin is a key molecule in regulation of endothelial integrity (Dejana et al., 1999). It is a target for several permeability-inducing agents, and downregulation of VE-cadherin leads to transient vascular permeability and paracellular movement of solutes and cells across the endothelium (Corada et al., 1999; Gao et al., 2000). Also important is the actin-based cytoskeleton, which is anchored to the junctional proteins and can exert contractile forces that change the cellular morphology and disrupt IEJs (Majno et al., 1969; Morel et al., 1990). These cytoskeletal changes are regulated by phosphorylation of myosin light chain by Ca^{2+}/calmodulin-dependent myosin light chain kinase or activation of Rho/Rho kinase (Stevens et al., 2000; Dudek and Garcia, 2001; Rigor et al., 2013). Vascular permeability can also be controlled via transcellular pathways that involve caveolae-mediated endocytosis and vesicular trafficking, although it is unclear if sphingolipids play a significant role in this pathway (Predescu and Palade, 1993; Gavard and Gutkind, 2006; Zhang et al., 2014). As the understanding of these pathways has developed, the sphingolipids have surfaced at multiple points as inducers, inhibitors, and regulators of vascular permeability.

Sphingosine-1-phosphate

Sphingosine-1-phosphate is a pleiotropic, bioactive lipid that has been well studied as a key mediator in the vasculature. It is the most potent intercellular signaling molecule in the sphingolipid family. Its structure as a lysophos-pholipid, with its hydrophobic tail and polar head, allows S1P to leave the plasma membrane and function in intracellular signaling via G protein-coupled receptors (Obinata and Hla, 2012). Additionally, several S1P transporters, including the ATP-binding cassette transporters and spinster homolog 2 (SPNS2), are expressed in vascular endothelial cells and facilitate release of S1P into the extracellular space where it can have multiple effects (Nishi et al., 2014). In one study, mice that were deficient in SPNS2 demonstrated decreased plasma levels of S1P and impaired lymphocyte egress from secondary lymphoid tissues (Hisano et al., 2012). The SPNS2 transporter also interacts with multiple S1P metabolic enzymes and signaling pathways and may have important effects on cancer cell survival and migration (Bradley et al., 2014). There are five different G protein-coupled receptors that bind S1P ($S1P_1$–$S1P_5$) (Lucke and Levkau, 2010). Endothelial cells express the $S1P_1$ receptor most abundantly, with the $S1P_2$ and $S1P_3$ receptors also expressed to a lesser degree (Wilkerson and Argraves, 2014). These receptors are differentially expressed by varying types of endothelial cells as well as in different tissue types, accounting for much of the complexity of the relationship between S1P and the endothelium.

Within the endothelium, S1P plays vital roles in angiogenesis, endothelial cell proliferation and migration, and vascular permeability. Double-knockout mice deficient in SphK1 and SphK2 result in embryonic lethality due to abnormal vascular development, proving a vital and necessary function of S1P (Xiong et al., 2014). Alternatively, knockout mice deficient in only one sphingosine kinase show no embryologic or developmental abnormalities, again

demonstrating the complexity and overlap in the relationship between S1P and the endothelium (Kono et al., 2004).

Breakdown of the normal endothelial cell barrier is pathologic in a wide array of disease processes, from acute lung injury to tumor angiogenesis, and normal plasma S1P levels are vital to maintain an intact endothelial barrier. Both *in vivo* and *in vitro* studies support the role of S1P in maintenance of normal endothelial barrier function. Mice deficient in S1P have increased vascular permeability and mortality after induction of anaphylaxis by exposure to histamine and PAF (Camerer et al., 2009). The integrity of the endothelial barrier in S1P-deficient mice was restored by transfusion of erythrocytes from wild-type animals with normal, erythrocyte-derived S1P stores. In an animal model of thrombin-induced vascular leak, S1P concentration and SphK1 activity are both increased; in mice deficient in SphK1 and S1P generation, both thrombin and LPS treatment cause significantly increased pulmonary edema (Tauseef et al., 2008). In human umbilical vein endothelial cells (HUVECs) grown in culture, addition of physiologic levels of S1P increases the transendothelial electrical resistance, a measurement of cellular barrier function (Schaphorst et al., 2003; Li et al., 2015). These findings support that S1P in the plasma is required to maintain normal endothelial barrier function. As the half-life of circulating S1P is rapid (< 15 min), S1P is likely continually produced by hematopoietic cells, including activated platelets and erythrocytes. In addition, production and release of S1P by endothelial cells can act as a local stimulus on endothelial cells to affect barrier function (Venkataraman et al., 2008). Administration of exogenous S1P prevented endothelial apoptosis and gastrointestinal syndrome in mice exposed to high-dose radiation, an effect that was observed *in vitro* and *in vivo* (Bonnaud et al., 2010). This effect was not observed in intestinal epithelial cells or lymphocytes, suggesting that these effects may be specific to the endothelium.

The mechanism of S1P-induced barrier protection is multifactorial, and of the five receptors, the $S1P_1$ receptor subtype appears to play the most critical role in this process. $S1P_1$ receptor ligation by S1P induces rearrangement of the endothelial cell cytoskeleton by formation of strong cortical actin rings to increase stability of cell-to-cell contact. When bound, the $S1P_1$ receptor induces Rac1, a small GTPase that increases actin polymerization at the cell periphery, augmenting endothelial cell junctional integrity and barrier strength (Garcia et al., 2001). Rac1 activation by S1P also immediately recruits other factors, including c-Abl and non-muscle myosin light chain kinase, to lipid rafts and to the endothelial cell periphery. Activation of these cytoskeletal signaling molecules as well as increased lamellipodia formation and protrusion at the cell periphery both increase cell overlap and augment endothelial cell-to-cell barrier (Garcia et al., 2001; Adyshev et al., 2011). Another downstream target of Rac activation, p21-associated Ser/Thr kinase (PAK), results in inactivation of cofilin, a protein responsible for actin disassembly (Horiuchi and Mogi, 2011). S1P-mediated activation of Rac results in enhancement of barrier function through this mechanism, and both blockade of PAK and overexpression of cofilin attenuate S1P-mediated barrier-protective effects. Rac activation by S1P also translocates the actin-binding protein cortactin to the cellular periphery, which induces additional actin polymerization and stabilization and further strengthens the endothelial cell barrier (Singleton et al., 2005; Belvitch and Dudek, 2012).

When bound to $S1P_1$, S1P also increases the strength of the endothelial cell barrier by inducing assembly of AJ and TJ proteins. Supplementation of S1P to cultured endothelial cells increases the interaction of VE-cadherin with β-catenin, increasing stability from AJ proteins by binding to the actin cytoskeleton (Bazzoni and Dejana, 2004; Hla and Brinkmann, 2011). Stimulation of endothelial cells by S1P also induces reassignment of zona occludens protein 1 (ZO-1) to paracellular junctions, which has a stabilizing effect on endothelial tight junctions (Lee et al., 2006). When ZO-1 expression is downregulated, S1P-mediated endothelial barrier enhancement is compromised, affirming the role of S1P in tight junctions. The critical role of $S1P_1$ receptor in endothelial cell barrier integrity is corroborated by *in vivo* blockade of $S1P_1$ receptor in a model of VEGF-induced pulmonary edema. Blockade of the receptor results in enhanced pulmonary vascular leak in mice, and $S1P_1$ receptor knockouts are lethal *in utero* due to hemorrhage from vessel rupture (Allende et al., 2003; Schuchardt et al., 2011).

Compared to the $S1P_1$ receptor, less is known regarding the $S1P_2$ and $S1P_3$ receptors. Binding of the $S1P_2$ receptor by S1P inhibits normal protective endothelial barrier function by activation of RhoA-mediated signaling, which works in opposition to the activation of Rac (Skoura and Hla, 2009). When $S1P_2$ receptors are expressed in cultured HUVECs, the cortical actin and lamellipodia required to maintain normal endothelial barrier function are inhibited. Higher concentrations of S1P can activate $S1P_2$ and $S1P_3$ receptors preferentially and stimulate RhoA, which increases stress fiber formation and cellular contractility, inducing vascular leak (Adyshev et al., 2011). Downstream mediators of RhoA activation include Rho-associated protein kinase 1, which inactivates myosin light chain phosphatase resulting in disarray of tight junction proteins, and PTEN (phosphatase and tensin homolog deleted on chromosome 10), which also increases paracellular permeability (Sanchez et al., 2007). In addition, $S1P_2$ activation disrupts AJs by inhibiting VE-cadherin, which increases vascular leak by increasing paracellular permeability (Sanchez et al., 2007; Skoura and Hla, 2009). The functionality of the $S1P_3$ receptor also is thought to oppose the protective effects of the $S1P_1$ receptor in a similar fashion. In *in vitro* studies of intratracheally administered S1P, activation of $S1P_3$ receptor on pulmonary epithelium induces pulmonary edema by RhoA-mediated signaling pathways similar to the $S1P_2$ receptor (Obinata and Hla, 2012). $S1P_3$ receptor is also implicated in thrombin-induced endothelial barrier disruption, mediated by RhoA transactivation (Spindler et al., 2010). In most studies, S1P preferentially activates $S1P_1$ over $S1P_2$ and $S1P_3$ to preserve the normal barrier function of the endothelium, although there is some evidence to suggest that this may be a concentration-dependent effect with $S1P_1$ activated at physiologic S1P concentrations and $S1P_2$ and $S1P_3$ activated by excessive concentrations (Li et al., 2015).

The interaction between S1P and activated protein C (APC), another potent regulator of endothelial cell permeability, has the potential to further augment barrier function. Protein C becomes activated when it is bound to the endothelial protein C receptor (EPCR) and the APC-EPCR complex then inhibits thrombin-mediated vascular leak (Finigan et al., 2005). The APC-EPCR complex exerts its protective effect by activation of protease-activated receptor 1 (PAR1) as well as by activation of Rac1. Induction of Rac1 by the APC-EPCR complex results in similar cytoskeletal rearrangements that are described with binding of S1P to the $S1P_1$ receptor, and both processes result in endothelial barrier protection. Newer

evidence also supports the transactivation of $S1P_1$ by the APC-EPCR complex. Blockade of EPCR eliminates the activation of $S1P_1$, supporting this theory, and silencing $S1P_1$ receptors effectively negated APC-mediated endothelial barrier protection in a model of thrombin-induced vascular leak (Finigan et al., 2005). Although the mechanism is unclear, this evidence supports significant cross talk between EPCR and $S1P_1$ receptors to augment endothelial cell barrier function.

In addition to the complex and overlapping effects of S1P on the endothelial cell cytoskeleton, S1P plays a vital role in inflammation and lymphocyte trafficking. S1P results in a profound reduction of circulating lymphocytes but does not affect activation, proliferation, or maturation of these cells (Obinata and Hla, 2012). Instead, S1P affects mobilization of lymphocytes, specifically T cells, from the thymus and lymphoid tissues. S1P concentrations are significantly higher in the plasma and lymph as compared to tissue concentrations, and lymphocytes take advantage of this ‘S1P gradient’ to exit tissue (Wilkerson and Argraves, 2014). Similarly, natural killer cells, hematopoietic stem cells, and dendritic cells use this signaling system for mobilization. If the S1P gradient is disrupted, i.e., with inhibition of S1P lyase or the sphingosine kinases, the number of circulating inflammatory cells decreases while overall numbers are not altered, supporting the role of S1P in mobilization of leukocytes (Schwab et al., 2005; Pappu et al., 2007). Once inflammatory cells are mobilized, S1P also affects the interaction between leukocytes and the endothelium. Addition of S1P inhibits adhesion molecule expression, disrupting the ability of inflammatory cells to adhere to the endothelium.

Ceramide

The role of ceramide in endothelial function is not as well established as that of S1P, but ceramide is a known mediator of multiple eukaryotic stress responses. It is associated with barrier disruption and increased permeability and can be generated at high levels in the endothelial cell membrane. Vascular endothelial cells have baseline levels of ASM expression up to 20-fold higher than other cell types and can increase secretion an additional 2- to 3-fold after activation by inflammatory cytokines (Marathe et al., 1998). Once formed, ceramide has at least two general mechanisms by which it may alter endothelial permeability. One mechanism of action is as a messenger in intracellular signaling pathways. Acid sphingomyelinase is activated by numerous cellular stressors, such as TNFα, CD95/Fas/APO-1, ionizing radiation, UV-C, and oxidative stress, resulting in a rapid increase in production of ceramide. Ceramide then acts as a second messenger with multiple potential downstream targets (Gulbins, 2003). Ceramide has been shown to activate the protein phosphatases PP1 and PP2A, the lysosomal protease cathepsin D, kinase suppressor of Ras, and protein kinase C-zeta (PKCζ) (Hannun and Obeid, 2002). This may be through direct action (PP1 and PKCζ) or action on intermediaries such as inactivation of the PP2A inhibitor SET (Maceyka and Spiegel, 2014). In addition to its role as a second messenger, ceramide may exert effects on the endothelium by self-associating to form ceramide-enriched rafts within the cell membrane. These domains can trap signaling receptors and alter the biophysical properties of the membrane, leading to amplified or targeted receptor responses (Henry et al., 2013). This variety of targets and effectors helps explain the diverse

effects attributable to ceramide, such as regulation of apoptosis, capillary leak, and cytoskeletal remodeling.

The role of ceramide as a mediator of apoptosis is well established (Yao et al., 1995; Haimovitz-Friedman et al., 1997b; Mathias et al., 1998). Elevated ceramide levels are associated with pulmonary cell apoptosis and development of emphysema (Petrache et al., 2005, 2011). Likewise, Haimovitz-Friedman and colleagues (1997a) demonstrated that ceramide generation, stimulated by TNFα, leads to disseminated endothelial apoptosis and endotoxic shock syndrome after exposure to LPS. Ceramide has also been demonstrated as a second messenger in endothelial apoptosis stimulated by radiation (Lin et al., 2000). Several cellular systems have shown the ability of ceramide to activate the SAPK/JNK cascade, as well as ceramide-mediated apoptosis that occurs downstream from the Fas-associated death domain protein complex and IL-1β-converting enzyme/Ced3 pathway (Haimovitz-Friedman et al., 1997b). Additionally, multiple stressors have shown activation of apoptosis via ceramide-dependent activation of BCL-2 family proteins (Zhang and Saghatelian, 2013). Thus, ceramide-induced apoptosis has been considered as a potential mechanism for ceramide's role in vascular permeability. However, several studies conducted over the past decade have downplayed apoptosis as the mechanism of ceramide-mediated endothelial permeability and focused on alternate or additional mechanisms.

Goggel and coworkers (2004) initially showed a link between PAF and ceramide in the formation of pulmonary edema in which PAF activated ASM. This effect on vascular permeability and edema formation was demonstrated to be independent of cell necrosis or apoptosis (Lindner et al., 2005). This led to the elucidation of two signaling pathways involving activation of ASM and formation of ceramide in formation of lung edema. In the first, activation of ASM leads to caveolin-1 accumulation in caveolae and a resultant drop in endothelial nitric oxide synthase (eNOS) activity and decreased endothelial nitric oxide (eNO) production, leading to formation of edema (Yang et al., 2010). In a second pathway, PAF stimulates ASM and leads to recruitment of transient receptor potential classical 6 to caveolae, resulting in Ca^{2+} entry into the cell and activation of myosin light chain kinase (Samapati et al., 2012). Interestingly, elevated ceramide leads to loss of endothelial integrity and edema in the systemic circulation as well, but via a different mechanism than that seen in pulmonary circulation (Kuebler et al., 2010). Ceramide leads to activation of eNOS and elevated eNO in systemic endothelial cells with formation of larger paracellular gaps than those seen in the pulmonary circulation and the same end result of interstitial edema (Igarashi et al., 1999; Czarny and Schnitzer, 2004). The exact reasons for this divergent effect are unclear, but may be related to differences in caveolar organization and function and to the unique endothelial cell subtypes expressed in different tissues (Kuebler et al., 2010).

Other potential mechanisms by which ceramide may influence endothelial permeability include oxidative stress and cytoskeletal modification. There is evidence that increased ceramide in vascular tissues leads to impairment of endothelial-dependent vasodilation (Zheng et al., 2000). This endothelial dysfunction is closely associated with increased formation of reactive oxygen species and decrease in eNO (Zhang et al., 2001). Ceramide has also been shown to induce RhoA- and redox-dependent morphological changes and

cytotoxicity in endothelial cells (Gupta et al., 2001). Schweitzer and colleagues (2011) showed that cigarette smoke triggered activation of neutral sphingomyelinase leading to ceramide-dependent cytoskeletal changes and endothelial permeability that involved p38 MAPK, JNK, oxidative stress, and Rho kinase activation. Additionally, recent findings by Kolliputi et al. (2012) showed that ceramide can induce release of proinflammatory cytokines and epithelial permeability in the lung via inflammasome activation. However, it remains unclear if endothelial disruption may also occur via this pathway.

Examples from specific disease processes

As the role of bioactive lipids in the pathogenesis of numerous disease processes becomes clearer, two clinical syndromes in particular stand out in which the interplay between sphingolipids (notably ceramide and S1P) and endothelial cells has emerged as a driving force. Disruption of the endothelial barrier is a hallmark feature of both sepsis and acute lung injury, and there is increasing evidence that disruption of lipid homeostasis is a critical step in the pathogenesis of both of these disease processes.

Sepsis

Sepsis is a systemic response to infection and inflammation marked by disseminated endothelial dysfunction, labile blood pressure, coagulopathy, and end organ failure. Ceramide acts as a second messenger for the proinflammatory cytokines TNF-α and IL-1β (Kolesnick and Golde, 1994). In the presence of infection, bacterial LPS may increase levels of ceramide and potentiate its systemic inflammatory effects (MacKichan and DeFranco, 1999). However, ceramide is also structurally and functionally similar to LPS, and even in the absence of bacterial infection ceramide may lead to a sepsis-like syndrome by binding the LPS receptor CD14 and inducing clustering and activation of co-receptors in lipid rafts (Wright and Kolesnick, 1995; Pfeiffer et al., 2001). Clinically, ceramide levels are increased in the serum of patients with sepsis and the increase is associated with increased multiple organ dysfunction and mortality (Delogu et al., 1999; Drobnik et al., 2003). In a prospective study, Claus and coworkers (2005) found increased sphingomyelin phosphodiesterase 1 (SMPD1, the gene responsible for formation of ASM) activity in the plasma of septic patients that correlated directly with severity of illness and mortality, as well as improved survival after inhibition of SMPD1 in a murine model of sepsis. The role of S1P in sepsis is less clear. Drastic changes in lipoprotein levels and metabolism can occur in patients with severe sepsis, with decreases in HDL and apolipoproteins (apoA1, apoB, and apoM) (van Leeuwen et al., 2003; Barlage et al., 2009). ApoM is a carrier protein that links S1P to HDL in the circulation, and lower levels of apoM are associated with more severe disease in patients with sepsis and systemic inflammatory response syndrome, suggesting that the loss of S1P may be involved in the vascular permeability and morbidity seen in sepsis and related syndromes (Kumaraswamy et al., 2012).

Acute lung injury

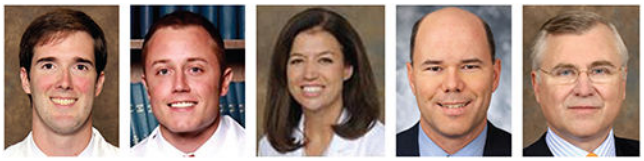
Animal models have shown that ASM activity and ceramide formation are increased with lung injury induced via multiple different mechanisms (Goggel et al., 2004; von Bismarck et al., 2007, 2008). Treatment with intravenous S1P was associated with decreased edema

formation and inflammatory cell recruitment in isolated perfused rat lungs and in murine and canine models of lung injury (Goggel et al., 2004; McVerry et al., 2004; Peng et al., 2004). Likewise, treatment with intravenous S1P or an analogue decreased lung injury in a murine pancreatitis model (Liu et al., 2008). In a separate set of experiments, inhibition or knockout of S1P lyase, an enzyme that irreversibly degrades S1P, led to increased levels of S1P in bronchoalveolar lavage fluid and decreased lung injury in a murine model of inflammatory lung injury (Zhao et al., 2011). Sammani et al. (2010) further explored the contribution of three S1P receptors to lung injury and edema and found that both intravenous S1P and a selective $S1P_1$ agonist were protective of inflammatory lung injury. Treatment with an inverse agonist of $S1P_1$ or knockout of one $S1P_1$ allele attenuated the protective effect, while silencing the $S1P_2$ and $S1P_3$ isoforms was lung protective (Sammani et al., 2010). Of note, in the same study, intratracheal delivery of S1P and $S1P_1$ agonist had a detrimental effect, leading to inflammation, disruption of the alveolar capillary barrier, and death. These findings have yet to be corroborated with clinical data, but there is mounting evidence implicating sphingolipids as mediators of lung injury and pulmonary edema.

Future directions

The potential for sphingolipids as therapeutic targets has already been realized in several settings. Enzyme replacement therapy is a well-established therapy for multiple lysosomal storage disorders, and the sphingosine analogue FTY720 is routinely used for treatment of the relapsing/remitting type of multiple sclerosis (Futerman and van Meer, 2004; Jmoudiak and Futerman, 2005; Halmer et al., 2014). Increased understanding of sphingolipid metabolism and the increasing availability of sphingolipid modulators have opened the door to new therapeutic avenues, including treatment for disease processes where the endothelium plays a key role in pathogenesis. However, one particular area of concern going forward is the potential for systemic side effects. The ubiquitous nature of many sphingolipids and the complexity of sphingolipid pathways could lead to unintended consequences in the setting of therapeutic modification. However, several existing non-specific therapies, such as FTY720 and the ASM-inhibiting tricyclic antidepressants, are generally well tolerated by patients with limited systemic side effects (Beckmann et al., 2014). Furthermore, the discovery and increased understanding of new targets in sphingolipid pathways such as ceramide 1-phosphate, glucosyl sphingolipids, and specific receptor isoforms will help to develop more directed treatment strategies. Given the important role that sphingolipids play in regulating endothelial barrier function and the wide-ranging pathology that develops with endothelial disruption, sphingolipids represent likely targets for novel therapeutics with wide-ranging applications.

Bionotes



From left to right: Peter L. Jernigan and Richard S. Hoehn are surgical residents at the University of Cincinnati College of Medicine currently participating in a research fellowship. Their areas of research interest include blood banking, trauma, and sphingolipid biology. Amy T. Makley and Timothy A. Pritts are trauma surgeons at the University of Cincinnati with research interests including blood banking, hemor-rhagic shock and resuscitation, and lung injury. Michael J. Edwards is a surgical oncologist and the chairman of surgery at the University of Cincinnati with specific research interests including sphingolipids and translational medicine.

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