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## SPHINGOSINE 1-PHOSPHATE AS A THERAPEUTIC TARGET IN HEART FAILURE: MORE QUESTIONS THAN ANSWERS

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Sphingosine-1-phosphate (S1P) is a naturally occurring bioactive lysophospholipid that regulates immune responses and inflammatory processes in a variety of different organ systems, including the cardiovascular system. Within the cardiovascular system S1P mediates cardioprotection following ischemia/reperfusion injury, cardiac remodeling, vascular tone, angiogenesis, as well as fibroblast migration, proliferation, and differentiation (reviewed in reference <sup>1</sup>). In the current edition of *Circulation*, Meissner and colleagues present novel findings in a murine heart failure model (LAD ligation) which suggest that S1P activity is modulated by a cAMP-responsive chloride channel termed the cystic fibrosis transmembrane regulator (CFTR).<sup>2</sup> CFTR, a member of the ATP binding cassette family of genes, was identified as the gene responsible for the loss of chloride secretion in patients with cystic fibrosis. Based on a prior report by the same group,<sup>3</sup> which showed that the intracellular enzyme S1P phosphohydrolase that degrades extracellular S1P, was also an endogenous regulator of S1P mediated vasoconstriction, the authors hypothesized that S1P must be imported by vascular smooth muscle cells. In their current report Meissner et al. show that CFTR mRNA was present in conduit and resistance arteries throughout the vascular tree in mice. They further noted that CFTR was required for S1P uptake by vascular smooth muscle cells, and that VSMCs from wild-type (CFTR<sup>+/+</sup>) possessed a higher rate of proliferation than VSMCs from CFTR null (CFTR<sup>-/-</sup>) mice, consistent with the known anti-proliferative effects of S1P on VSMCs. At the whole organ level, S1P mediated vasoconstriction of small resistance arteries (e.g. the posterior cerebral artery) was more potent in CFTR<sup>-/-</sup> than in CFTR<sup>+/+</sup> mice, whereas the vasoconstrictor response to phenylephrine, and the vasodilator response to acetylcholine did not differ between CFTR<sup>-/-</sup> and CFTR<sup>+/+</sup> mice. Finally the authors showed that CFTR mRNA and levels were decreased in resistance vessels of wild-type mice that had undergone acute LAD ligation, suggesting that decreased uptake of S1P of CFTR resulted in sustained S1P signaling and enhanced vascular tone. The authors further made the interesting observation that CFTR mRNA levels were downregulated by tumor necrosis factor (TNF), a pro-inflammatory cytokine that is increased in heart failure,<sup>4</sup> and that treatment with etanercept, a TNF antagonist,<sup>5</sup> rescued many aspects of the heart failure phenotype, including normalization of vascular tone, as well as downregulation of CFTR. Given that the CFTR downregulation was reversible, and

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### Conflict of Interest Statement

The author has no conflict of interest relevant to this manuscript.

that increased peripheral vasoconstriction contributes to disease progression in heart failure, Meissner et al. suggest that CFTR may represent a novel target for cardiovascular conditions such as heart failure, wherein inflammation is present. Before addressing this interesting question, it is instructive to first review sphingosine-1-phosphate signaling within the cardiovascular system.

## Sphingosine-1-Phosphate Signaling in Cardiovascular Disease

Sphingosine-1-phosphate (S1P), which was once regarded as a simple intermediate of sphingolipid metabolism, is now recognized as a critical regulator of a broad variety of pathophysiological processes, including atherosclerosis, diabetes, osteoporosis, multiple sclerosis, Alzheimer's disease and cancer. S1P is formed within cells by phosphorylation of sphingosine, which is a backbone component of all sphingolipids, by the action of two isoenzymes of sphingosine kinase, SphK1 and SphK2, which differ in their catalytic properties, subcellular location, and tissue distribution. S1P not only functions as an external ligand for G-protein coupled receptors after it is secreted by cells (inside-out-signaling), but it can also act as an intracellular second messenger, as discussed below. Most of the known actions of S1P are mediated by binding of secreted S1P to a family of five G protein-coupled receptors, termed S1P<sub>1-5</sub>. Thus far, S1P<sub>1-3</sub> receptors have been detected in the heart. In the heart S1P<sub>1</sub> mediated signaling inhibits cAMP formation, and antagonizes adrenergic-mediated contractility through the Gi pathway.<sup>6</sup> The S1P/S1P receptor axis has also been shown to be important in terms of controlling vascular permeability through activation of S1P<sub>1</sub> (which decreases vascular permeability),<sup>7</sup> as well as vascular tone through S1P<sub>3</sub> activation.<sup>8</sup> S1P also has been shown to have important intracellular targets, including histone deacetylases, protein kinase C $\delta$ , TNF receptor-associated factor 2, and prohibitin 2, a highly conserved protein that regulates mitochondrial assembly and function.<sup>9</sup> Viewed together, these observations suggest that S1P signaling exerts a broad array of different extracellular and intracellular effects, which has important implications for the development of targeted therapeutics of the S1P/S1P axis.

The rapid expansion of knowledge with regard to the biology of S1P signaling, has sparked interest in developing therapeutic agents to modulate the S1P/S1P receptor signaling axis. Nonetheless, despite the proliferation of the S1P agonists/antagonists (see Table), very little is known with respect to the effects of these pharmacologic agents in the context of cardiovascular disease. Germane to the present discussion, AUY954 is a selective S1P<sub>1</sub> agonist that has been shown to prevent allograft rejection in a rat cardiac transplantation model.<sup>10</sup> FTY720 (Fingolimod) is an orally available S1P receptor agonist, approved by the FDA for the treatment of multiple sclerosis, that induces functional change in lymphocytes and macrophages. Remarkably, FTY720 has been shown to significantly reduce atherosclerotic plaques in apoE deficient mice.<sup>11</sup> FTY720 has also been shown to also prevent ischemia reperfusion induced cardiac arrhythmias through enhanced Akt signaling,<sup>12</sup> consistent with prior studies in S1P<sub>2,3</sub> receptor knockout mice that demonstrated an increase in infarct size following ischemia reperfusion injury.<sup>1</sup> Although prior studies have not attempted to modulate S1P levels by modulating the CFTR chloride channel, there has been tremendous interest in chloride channels as drug targets because their role in cystic fibrosis and other human diseases.<sup>13</sup> In this regard it is worth noting that patients with cystic

fibrosis have a cardiac phenotype that is characterized by a preserved ejection fraction with abnormalities of relaxation.<sup>14</sup> Recently, ivacaftor (Kalydeco™), which works by increasing the open probability of mutant CFTR channels, was shown to improve lung function in patients with cystic fibrosis,<sup>15</sup> and was recently FDA approved for the treatment of this orphan indication. Of note, the current generation of CFTR potentiators do not increase the activity of normal (wild-type) CFTR channels, which are already maximally activated by cAMP.<sup>13</sup> Accordingly, these agents may be less useful for modulating S1P levels in situations where the total protein content is reduced.

### **Is sphingosine-1-phosphate signaling a therapeutic target in heart failure?**

Unfortunately, at the time of this writing it is unclear whether one would want to augment or diminish S1P signaling in patients with heart failure. The results of the carefully done study by Meissner and colleagues<sup>16</sup> suggest that sustained S1P signaling secondary to decreased CFTR mediated uptake of S1P may be deleterious because of enhanced microvascular tone in small resistance arteries. While it is certainly true that increased peripheral vascular resistance contributes to functional limitations in heart failure patients, as well as progressive pump dysfunction in advanced heart failure, it is unclear whether the increase in vascular tone in small resistance arteries (mesenteric, cremasteric and posterior cerebral artery) is also present in the systemic arteries that are responsible for the increased peripheral vascular resistance in heart failure. Indeed, whereas S1P constricts small arteries in the mesentery, cerebral and renal arteries, S1P has no effect on the aorta, carotid or femoral arteries, and S1P signaling has been associated with vasodilation in various studies in small and large arteries, which is likely related to the unique distribution of S1P receptors in different vascular beds.<sup>8</sup> Moreover, we have no information on what happens to S1P receptors in the peripheral vasculature of patients with heart failure. It is also important to recognize that S1P levels are not elevated in the peripheral circulation of patients with symptomatic heart failure, suggesting that the S1P/S1P receptor axis may not be dysregulated in heart failure.<sup>17</sup> Finally, as noted above the extant literature suggests that enhanced S1P signaling is generally beneficial in the setting of acute cardiac injury. Thus, at the time of this writing, there simply is insufficient information to suggest that strategies that either upregulate or downregulate sphingosine-1-phosphate signaling will be effective in the setting of heart failure. Nonetheless, one of the qualities of important research is that it frequently stimulates more questions than can be answered in a single study. Given the proliferation of pharmacologic agents that are capable of modulating S1P signaling at a variety of different levels, including S1P antagonists/agonists, as well as inhibitors of S1P synthesis/degradation (Table) the provocative questions raised in the important study by Meissner and colleagues<sup>16</sup> are likely to stimulate a number of laboratories to focus future research efforts on this burgeoning area of cardiovascular biology.

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**Table**

## Small Molecule Inhibitors of the S1P/S1P Receptor Signaling Axis

Agonist/antagonist	Target	Animal studies	Clinical studies
FTY720 (Fingolimod)*	S1P <sub>1/3/4/5</sub>	Yes	Yes
AAL-R	S1P <sub>1/3/4/5</sub>	ND	ND
KRP-203	S1P <sub>1</sub>	Yes	ND
AUY954	S1P <sub>1</sub>	Yes	Yes
RG3477	S1P <sub>1</sub>	Yes	Yes
SEW2871	S1P <sub>1</sub>	Yes	ND
CYM-5442	S1P <sub>1</sub>	Yes	ND
W146	S1P <sub>1</sub>	Yes	ND
Compound 5	S1P <sub>1</sub>	Yes	ND
JTE013	S1P <sub>2</sub>	Yes	ND
TY-52156	S1P <sub>3</sub>	Yes	ND
VPC23019	S1P <sub>1/3</sub>	ND	ND
VPC44116	S1P <sub>1/3</sub>	Yes	ND
SK1-1	SphK1	Yes	ND
Safingol	Sphk1	Yes	ND
SKI	Sphk1	Yes	ND
ABC294640	SphK2	Yes	Yes
DMS	Sphk1,2	Yes	ND
SK-II	SphK1	Yes	ND
Reservatrol	SphK1	Yes	Yes
LX2931	S1p lyase	Yes	Yes

(Modified with permission from references 8 and 19) (key: ND = not done; SphK = sphingosine kinase;

\* = FDA approved for the treatment of multiple sclerosis)