Sphingosine-1-Phosphate Receptor 1 Activation Enhances Leptomeningeal Collateral Development and Improves Outcome after Stroke in Mice

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Background: Development of collateral circulation after acute ischemic stroke is triggered by shear stress that occurs in pre-existing arterioles. Recently, sphingosine-1-phosphate receptor 1 (S1P1) on endothelial cells was reported to sense shear stress and transduce its signaling pathways. *Methods:* BALB/c mice (n = 118) were subjected to permanent middle cerebral artery occlusion (pMCAO) or sham operation. We investigated the effect of an S1P1-selective agonist SEW2871 on leptomeningeal collateral arteries and neurological outcome after pMCAO. *Results:* Immunohistochemistry showed that without treatment, the expression of S1P1 on endothelial cells of leptomeningeal arteries and capillaries increased early after pMCAO, peaking at 6 hours, whereas a significant increase in the expression of S1P1 in neurons was seen from 24 hours later. After intraperitoneal administration of SEW2871 for 7 days after pMCAO, the number of leptomeningeal collateral arteries was significantly increased, cerebral blood flow improved, infarct volume was decreased, and neurological outcome improved compared with the controls. Significantly increased phosphorylation of endothelial nitric oxide synthase (eNOS) as early as 6 hours after pMCAO and higher expression of tight junction proteins at postoperative day 3 were observed with SEW2871 treatment as assessed by Western blot. Daily administration of SEW2871 also increased capillary density in peri-infarct regions and promoted monocyte/macrophage mobilization to the surface of ischemic cortex at 7 days after pMCAO. *Conclusions:* An S1P1-selective agonist enhanced leptomeningeal collateral circulation via eNOS

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Author contributions: E.I. designed the experiments; performed animal modeling, qRT-PCR, Western blotting, and statistical analyses; and drafted the manuscript. S.I. was involved in methodology, confocal microscopy, analysis, experimental design, and supervision. M.S. participated in immunostaining, confocal microscopy, and neurological assessment. F.Y.L. participated in cell culture, animal modeling, and qRT-PCR. M.I. and K.M. participated in conceptualization, provision of resources, and supervision. T.Y. is the last author and participated in conceptualization and supervised the project.

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phosphorylation and promoted postischemic angiogenesis with reinforced blood–brain barrier integrity in a mouse model of acute ischemic stroke, leading to smaller infarct volume and better neurological outcome. **Key Words:** Collateral circulation—ischemic stroke—leptomeningeal artery—shear stress—sphingosine-1-phosphate receptor 1 (S1P1).

Introduction

Stroke is the second leading cause of death worldwide[1] and its disease burden as measured by disability-adjusted life years is huge.[2] Ischemic stroke makes up 87% of all strokes.[3] Proximal intracranial artery occlusion is especially related to poorer prognosis.[4] In the setting of proximal middle cerebral artery (MCA) occlusion (MCAO), collateral circulation comes through leptomeningeal arteriolar connections from the anterior cerebral artery (ACA) to supply the superior or anterior division of the MCA and from the posterior cerebral artery to supply the posterior or inferior division of the MCA.[5] Development of collateral arteries has been associated with reduced infarct volume and better functional outcome in patients with ischemic stroke,[6] independent of the recanalization status of the occluded arteries.[7] Although leptomeningeal collateral enhancement before MCAO using granulocyte-macrophage colony-stimulating factor was shown to decrease infarct size in a mouse model of ischemic stroke,[8] no effective pharmacological intervention after MCAO has been developed to enhance collateral circulation.

Once hemodynamically relevant arterial stenosis or occlusion occurs, pre-existing arterioles redistribute the blood flow by connecting high-perfusion and low-perfusion regions, thus increasing the "shear stress" in the pre-existing arterioles. This shear stress triggers collateral vessel development in acute ischemic stroke.[9] In patients who underwent endovascular procedures for symptomatic stenosis of the proximal MCA, angiographic images revealed that within 15-30 seconds of balloon catheter inflation at the stenotic site, leptomeningeal arteries developed to maximally reconstitute the M1 segment just distal to the occluded point even in those who had few or no vessels seen before the procedure.[10] Thus, contrary to the belief that collateral vessels develop over time, collaterals can develop very quickly in response to shear stress.[11]

Sphingosine-1-phosphate (S1P) is a member of sphingolipid family of cell membrane–derived lipids and is involved in a variety of essential cellular processes such as cell growth, survival, motility, angiogenesis, trafficking of immune cells, and endothelial barrier integrity.[12,13] These multiple physiological functions of S1P are mediated by a family of G protein–coupled receptors, known as S1P receptor 1 (S1P1) through S1P5. S1P1 generally couples with the Gi subunit of heterotrimeric G proteins, which activates the Ras/extracellular signal-regulated kinase pathway, the phosphoinositide 2-kinase (PI3K)/protein kinase B (Akt) pathway, or the PI3K/Rac pathway to promote cell survival, cell proliferation, vasodilation, or cell migration. Vasodilation via endothelial nitric oxide synthase (eNOS) phosphorylation occurs as a downstream signal of the PI3K/Akt pathway.[12,14,15] In the central nervous system, S1P1 is expressed in neurons, glial cells, and endothelial cells,[12] although its expression level in each cell type may vary depending on the situation.[16-18] In rats with ischemic stroke, S1P1 expression is seen in neurons in the peri-infarct cortex 24 hours after MCAO.[18] In the setting of ischemic stroke, an S1P1 agonist (SEW2871 or LASW1238) and the S1P1 modulator fingolimod (FTY720) have been reported to be neuroprotective in rodents[18-21] and in human,[22] possibly acting through direct neuroprotection,[18,19] decreased immune cell activation,[19-21] or decreased microvascular permeability.[22] In addition to activating S1P1, S1P also activates S1P2, which destroys the blood–brain barrier and is thus neuroharmful in ischemic stroke conditions,[23] and S1P3, which is associated with reduced heart rate[24] and possibly a proinflammatory effect.[25] Thus, receptor-specific targeting is important.

Recently, S1P1 was reported to respond to shear stress and to transduce flow-mediated signaling in endothelial cells in vitro and in vivo in mouse descending aorta.[26] We have previously reported that S1P1 expression is upregulated in the leptomeningeal arteries under prolonged shear stress after occlusion of the ipsilateral common carotid artery. Under conditions of chronic shear stress and upregulated S1P1 in leptomeningeal arteries, an S1P1 agonist dilated the leptomeningeal collateral arteries of ACA-MCA anastomoses.[27] Still, in the setting of acute ischemic stroke, the role of S1P1 in leptomeningeal arteries is largely unknown.

Here, we investigated the distribution of S1P1 expression from the very acute stage of ischemic stroke, 6-48 hours after onset, in a mouse model of stroke. We hypothesized that S1P1 activation enhances the molecular response to shear stress, and thus leads to enhanced collateral circulation in ischemic stroke. We administered an S1P1-selective agonist daily to mice with surgically induced permanent MCAO (pMCAO) starting just after the occlusion, and analyzed cerebral blood flow (CBF) changes, diameters of collateral vessels, and neurological functions over 7 days.