Neuroprotective effects of vascular endothelial growth factor A in the experimental autoimmune encephalomyelitis model of multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by multiple demyelinating plaques in the white matter. For decades, the focus of MS research has been on inflammation-mediated demyelination of the white matter. However, recent studies suggest that neurodegeneration is not only an early event but also the primary cause of chronic disability in MS (Popescu and Lucchinetti, 2012). It is well documented that axon transaction and loss occurs early in the CNS of MS patients. A number of studies also reveal significant neuron loss in the CNS gray matter of MS patients, including the cerebral cortex, cerebellum, hippocampus, thalamus, and spinal cord. Moreover, magnetic resonance image studies show that progressive brain atrophy in MS patients correlates well with disability. Interestingly, both axon degeneration and neuron loss occur early in the CNS of animals undergoing experimental autoimmune encephalomyelitis (EAE), the primary animal model used in MS research (Stanojlovic et al., 2016). While it is generally believed that inflammation is responsible for neurodegeneration in MS and EAE, the mechanisms governing the viability of neurons and axons in these diseases remain largely unknown (Friese et al., 2014).

Vascular endothelial growth factor A (VEGF-A) was originally identified as an endothelial cell-specific growth factor, which stimulates angiogenesis and increases the permeability of blood vessels. Several lines of evidence have suggested that VEGF-A plays a role in various inflammatory diseases by enhancing angiogenesis and vascular permeability. Interestingly, recent studies show that VEGF-A also exerts direct actions on neurons and axons, and acts as a neurotrophic factor in the CNS under normal and disease conditions (Ruiz de Almodovar et al., 2009). The presence of VEGF-A in the CNS increases neuron survival and facilitates neurogenesis in various neurodegenerative diseases, such as amyotrophic lateral sclerosis, Alzheimer’s disease, Parkinson’s disease, spinocerebellar ataxia, and stroke. Moreover, there is evidence to suggest that VEGF-A is involved in the development of MS and EAE (Girolamo et al., 2014). Therefore, it is important to understand the effects of VEGF-A on neurodegeneration in MS and EAE.

VEGF-A exerts its function through several receptors, including VEGF receptor 1 (VEGFR1), VEGFR2, Neuropilin 1, and Neuropilin 2. It is believed that VEGF-A exerts direct actions on neurons and axons by binding to VEGFR2, resulting in autophosphorylation of the receptor and subsequent activation of its downstream signaling pathways (Carmeliet and Ruiz de Almodovar, 2013). In the last few years, a number of highly selective inhibitors of VEGFR2 have been identified. Among them, SU5416 (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidene]yl)-2-indolinone) is a potent VEGFR2 inhibitor and the first inhibitor to enter clinical trials for treatment of human diseases. Importantly, previous studies show that treatment with a low dose of SU5416 (10 mg/kg) attenuates the VEGF-A/VEGFR2 signaling in neurons and aggravates neuron death in mouse models of brain injury (Shimotake et al., 2010). Therefore, we sought to explore the role of the VEGF-A/VEGFR2 signaling in neurodegeneration in EAE mice by using SU5416.

While all major CNS cell types express VEGF-A, including neurons, astrocytes, oligodendroglia, microglia, and endothelial cells, previous data concerning the expression of VEGF-A in MS and EAE are contradictory. Some studies suggest that VEGF-A level is elevated in MS and EAE and that the elevated level of VEGF-A is associated with enhanced inflammation. In contrast, other studies show a decreased level of VEGF-A in these diseases (Girolamo et al., 2014). Therefore, we first measured the protein level of VEGF-A in the CNS of the well-characterized MOG35-55 EAE model using the highly sensitive and reproducible enzyme-linked immunosorbent assay (ELISA). When young adult female C57BL/6J mice are immunized with MOG35-55 peptide, the mice display neurological signs of disease starting as early as post-immunization day (PID) 12, reach the peak of disease around PID 19, and start recovering from EAE at around PID 22 (Lin et al., 2014). We found that VEGF-A level was not changed in the spinal cord of EAE mice at the onset of disease, but was significantly, although moderately, reduced at both the acute phase and chronic phase of EAE, compared to naïve mice (Stanojlovic et al., 2016). Nevertheless, we found that neither VEGFR2 level nor phosphorylated VEGFR2 level were significantly altered in lower motor neurons in the lumbar spinal cord of EAE mice compared to naïve mice (Stanojlovic et al., 2016).

Data indicate that the presence of VEGF-A in the CNS facilitates CNS inflammation in EAE mainly by increasing the permeability of the blood-brain barrier (BBB) through activation of VEGFR2 in endothelial cells (Girolamo et al., 2014). Our goal was to dissect the effects of the VEGF-A/VEGFR2 signaling on neurodegeneration during EAE. As described above, inflammation contributes to neurodegeneration in MS and EAE. Thus, to achieve our goal, we must minimize the impact of SU5416 treatment on inflammation. Interestingly, a number of studies show that the impact of the VEGF-A/VEGFR2 signaling on CNS inflammation is determined by the timing of activation. A previous study reports that treatment with a high dose of SU5416 (50 mg/kg) during the acute phase of EAE attenuates inflammation in the CNS; however, the same treatment during the chronic phase of EAE does not alter inflammation (Roscoe et al., 2009). Moreover, evidence suggests that neurons are more responsive to the alteration of the VEGF-A/VEGFR2 signaling than endothelial cells in the CNS. Additionally, it is known that BBB breakdown and inflammatory cell infiltration occur in the CNS of EAE mice well before the onset of clinical symptoms (Lin et al., 2013). Therefore, we decided to use a low dose of SU5416 (20 mg/kg) and started the treatment on the day after EAE disease onset. Importantly, we found that treatment with the low dose of SU5416 (20 mg/kg) starting after EAE onset did not significantly alter EAE disease severity or inflammation in the CNS (Stanojlovic et al., 2016).

A previous in vitro study shows that oligodendrocyte progenitor cells (OPCs) express VEGFR2 and that VEGF-A enhances OPC migration by activating VEGFR2. Nevertheless, there is no evidence that the VEGF-A/VEGFR2 signaling regulates the viability or function of mature oligodendrocytes under physiological or pathological conditions (Girolamo et al., 2014). It is well documented that oligodendrocyte death and myelin damage are caused by inflammation during EAE. Since treatment with the low dose of SU5416 (20 mg/kg) starting after EAE onset did not significantly alter inflammation in the CNS, it is not surprising...
that we found a minimal effect of SU5416 treatment on oligoden-
drocyte death and demyelination in the CNS of EAE mice. On the
other hand, in accordance with the previous studies (Shimotake et al., 2010), we found that treatment with the low dose of SU5416
(20 mg/kg) starting after EAE onset impaired VEGFR2 activation
in neurons in the CNS of EAE mice. Importantly, quantitative
immunohistochemistry analysis showed that SU5416 treatment
exacerbated lower motor neuron loss and axon loss in the lumbar
spinal cord of EAE mice. Nevertheless, SU5416 treatment had no
effect on Purkinje neuron loss in the cerebellum or upper mo-
tor neuron loss in the layer V of the cerebral cortex during EAE
(Stanojlovic et al., 2016). These data suggest that activation of the
VEGF-A/VEGFR2 signaling protects lower motor neurons and
axons in the spinal cord against inflammation during EAE, but
does not influences the viability of other neuron-types, such as
Purkinje neurons or upper motor neurons.

Evidence suggests that VEGF-A is an essential neurotrophic
factor for lower motor neurons in the spinal cord under phys-
iological conditions, and that activation of the VEGF-A/VEG-
FR2 signaling enhances lower motor neuron survival under
pathological conditions (Carmeliet and Ruiz de Almodovar,
2013). In agreement with these studies, our results revealed
the neuroprotective effects of the VEGF-A/VEGFR2 signaling
on lower motor neurons and axons in the spinal cord during EAE.
This study implies that therapeutic strategies that acti-
vate the VEGF-A/VEGFR2 signaling in neurons may be ben-
eficial to lower motor neurons and axons in the spinal cord of
MS patients.

Although our results derived from a pharmacological approach,
namely SU5416 treatment, provide the first evidence that the
VEGF-A/VEGFR2 signaling is involved in regulating the viability
of neurons and axons in animal model of MS, the approach has
several inherent shortcomings. First, it is impossible to rule out
the involvement of other CNS cell types in the protective effects
of the VEGF-A/VEGFR2 signaling on lower motor neurons and
axons in the spinal cord during EAE. All major CNS cell types
express VEGFR2, including neurons, astrocytes, oligodendroglia,
 microglia, and endothelial cells, and all these cells produce some
types of neurotrophic factors. There is a possibility that SU5416
treatment suppresses the production of neurotrophic factors in
non-neuronal cells via blockage of VEGFR2, resulting in exac-
erbation of neurodegeneration in EAE mice. Second, we cannot
exclude the contribution of the alteration of infiltration of inflam-
matory cells or expression of inflammatory mediators to the ex-
acerbated neurodegeneration in EAE mice treated with SU5416.
The molecular mechanisms by which inflammation causes neu-
rodegeneration in MS and EAE remain largely unknown. Many
inflammatory cells and inflammatory mediators are considered
as a double-edged sword in neurodegenerative diseases. SU5416
treatment may induce a subtle change in infiltration of inflamma-
tory cells or expression of inflammatory mediators via blockage
of VEGFR2 in endothelial cells or inflammatory cells, resulting
in exacerbation of neurodegeneration in EAE mice. Third, it is
unclear how SU5416 treatment exacerbates lower motor neuron
loss and axon loss in the spinal cord during EAE, but does not
affect the viability of Purkinje neurons or upper motor neurons.

Does the VEGF-A/VEGFR2 signaling selectively function in cer-
tain neuron types during EAE? Clearly, the protective role of
the VEGF-A/VEGFR2 signaling on neurons in MS warrants further
investigation. A genetic mouse model that allows for activation or
inactivation of VEGFR2 selectively in neurons would be an ideal
model to overcome the shortcomings mentioned above.

In summary, using a well-characterized, selective VEGFR2
inhibitor, our recent study indicates the neuroprotective role
of the VEGF-A/VEGFR2 signaling in the EAE model of MS.

Nevertheless, our mouse model system has its limits; additional
studies are essential and necessary to verify the cell autonomous
role of the VEGF-A/VEGFR2 signaling in neurons in the de-
velopment of MS and EAE. Additionally, there are many other
open questions regarding the role of VEGF-A in MS and EAE: 1)
cellular sources of VEGF-A; 2) the mechanisms regulating the
production of VEGF-A; 3) the effects of the VEGF-A/VEGFR2
signaling on other CNS cell types.

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