

Abstract

Hypoxia has been associated with multiple sclerosis (MS) and is an important area of research. Hypoxia can exacerbate inflammation via the prolylhydroxylase pathway. Inflammation can also trigger hypoxia by damaging mitochondria and endothelial cells to impair blood flow regulation. We hypothesize that there is a “hypoxia-inflammation cycle” in MS which plays an important role in MS disease progression. Therapies that break this cycle may be an interesting area of exploration for treatment of MS.

Introduction

Hypoxia has been associated with MS for years ¹. Type III lesions in MS brain are histologically similar to hypoxic/ischemic lesions, and lesions tend to form in areas that are more susceptible to hypoxia ². There is evidence for reduction in global metabolic rate in MS³, which could suggest that hypoxia/ischemia is not restricted to lesions, but rather a diffuse event. Inflammation is also distributed throughout the brain. Is there a link between hypoxia and inflammation?

Hypoxia may be critical in the context of MS. Signaling pathways for hypoxia and inflammation are intricately connected by the enzyme prolylhydroxylase (PHD).⁴ PHDs are responsible for breaking down HIF-1 α , the master regulator in the hypoxia response. Concurrently, PHD related pathways impact NF- κ B, which is involved in driving the production of pro-inflammatory cytokines. Hypoxia inhibits PHD, allowing both HIF-1 α as well as NF- κ B to become active.⁴ This not only triggers the up-regulation of genes involved in the hypoxia response, but also exacerbates the inflammatory response. Inflammation can damage the vascular endothelium, reduce vasoreactivity and promote the influx of leukocytes. An increased metabolic demand from

inflammatory cells may exceed the supply of oxygen, thus exacerbating hypoxia. Here, we review the evidence suggesting that diffuse hypoxia is present in MS, and propose that there is an “hypoxia-inflammation cycle” (Figure 1) that contributes to MS disease progression.

Presence of Hypoxia in MS

Accumulating evidence indicates that hypoxia is not restricted to the focal type III lesions, but is in fact a diffuse event. Using a rat experimental autoimmune encephalomyelitis (EAE) model of MS, it has been shown that partial pressure of oxygen (pO_2) in the spinal cord significantly decreased after induction of EAE.⁵ We used implanted pO_2 probes to quantify oxygen in the cerebellum and cortex of EAE mice and detected hypoxia compared to CFA controls.⁶ These results show that the EAE model of MS, an inflammatory model, shows significant levels of hypoxia.

Clinical studies also support the theory that hypoxia exists in patients with MS. Several groups have used MRI and showed that patients with MS have reduced cerebral blood flow (CBF) compared to controls, which is likely caused by impaired vasodilation. Impaired vasodilation⁷ and decreased CBF is significantly correlated with gray matter (GM) loss and cognitive impairment⁸, suggesting that insufficient blood flow (and thus hypoxic stress) may play an important role in neurodegeneration. These results are consistent with a hypoxia/ischemia induced metabolic stress, which ultimately lead to impairment of neuronal function.

We measured brain oxygenation in MS patients using near infrared spectroscopy (NIRS) and confirmed that approximately half of all MS patients have low microvascular hemoglobin

saturaton, consistent with gray matter hypoxia. NIRS is a non-invasive technique that utilizes the differential absorption properties of oxygenated and deoxygenated hemoglobin to calculate the tissue hemoglobin oxygen saturation (S_tO_2). We found that relapse-remitting patients with higher levels of impairment, as well as secondary progressive patients, had significantly lower S_tO_2 in their brains compared to controls.⁹ These evidence suggests that hypoxia is present in MS, and that it is relatively common phenomenon.

Inflammation drives hypoxia – evidence from other inflammatory diseases

Inflammation may be one of the key causes of hypoxia in many conditions, such as Crohn's disease, sepsis¹⁰, and the no-flow perfusion phenomenon seen in stroke.¹¹ Inflammation can cause hypoxia through several mechanisms. Reactive oxygen species (ROS) that are released during inflammation can damage the vascular endothelium and reduce the endothelial smooth muscles' ability to relax and cause vasodilation. This may be one of the mechanisms causing reduced CBF and hypoxia in MS. Using MRI to measure cerebral blood flow (CBF), it has been shown that vasodilation in response to CO_2 is significantly decreased in MS patients.

Inflammation can also cause physical obstruction of small blood vessels, a phenomenon that has been documented in ischemic stroke.¹¹ In experimental animal models of stroke, blood oxygenation levels in mice treated with IL-1 β continued to decline even after the reversal of the occlusion. Immunohistochemistry showed a significant increase in leukocytes, platelets and vascular adhesion molecules. Leukocytes and platelets aggravated in small blood vessels, which likely created physical ischemia in the microvasculature. It is possible that such events are occurring in patients with MS.

The idea that inflammation can cause physical obstruction of capillaries has been documented in the sepsis literature. It has been shown that administration of lipopolysaccharide (LPS) causes decreased cerebral blood flow in the microvasculature as well as cerebral tissue PO_2 .¹⁰ The decrease in tissue oxygenation is concurrent with a decrease in the proportion of small perfused vessels, which indicates that circulating inflammatory cells may be occluding small blood vessels.

The decrease in cerebral blood flow, combined with the influx of immune cells into the brain parenchyma may cause oxygen demand to far exceed the supply available, thus creating a hypoxic condition.

Hypoxia in MS: A detrimental phenomenon

The pathway initiating the hypoxic response is intricately connected with the inflammatory pathway through a class of oxygen sensing molecules called prolyl hydroxylases (PHDs).⁴ There are three types of PHDs (PHD-1, 2, 3), and they all require oxygen as a co-factor to function.⁴ Under normal levels of oxygen, PHDs prevents the stabilization of HIF1- α and inhibit the hypoxia response. PHDs under normal oxygen conditions also inhibit the NF- κ B (a master regulator of inflammation) by targeting the NF- κ B activation molecule Ikk β for degradation. When oxygen levels fall, PHDs are no longer active, resulting in activation of the HIF pathway as well as an accumulation of Ikk β , leading to the activation of NF- κ B.⁴ As NF- κ B is responsible for polarization innate and adaptive immune cells into a pro-inflammatory state, one could argue that hypoxia is detrimental and will exacerbate inflammation in MS.

In the context of MS, there is a large body of literature showing that NF- κ B activation is detrimental. Many of the new FDA approved MS medications, such as fingolimod, dimethyl fumarate, and teriflunomide all modulate the NF- κ B signaling pathway in some aspects.¹² NF- κ B is clearly important in the progression of MS, and measurement of tissue hypoxia may provide insights into the activity of this signaling pathway.

The idea that hypoxia can exacerbate inflammation in MS is supported by the observation that lesions are more common in areas that may be susceptible to hypoxia.^{2, 13, 14} Lesions are commonly formed at the lateral ventricles. Arterioles in this region are long and narrow, which could reduce the capacity to modulate flow and increase the susceptibility of these regions to hypoxic stress.¹⁴ Lesions are also commonly formed in areas known as watersheds, or borders of arterial territories. These areas have significantly lower perfusion, and have an increased susceptibility to hypoxia.¹³ ¹⁴ Others have also postulated that inflammatory events occurring in hypoperfused areas are likely to result in more damage, and are less likely to resolve.¹³

In summary, there is a growing body of evidence suggesting that hypoxic regions exist in the brain of some MS patients. It is difficult to determine whether hypoxia or inflammation is the primary event. However, it is likely that hypoxia exacerbates inflammation and vice versa, thereby creating a “hypoxia-inflammation cycle”. Thus, hypoxia could not only be a potential biomarker of pro-inflammatory activities, but also a therapeutic target.

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References

1. Gottlieb SF, Smith JE and Neubauer RA. The etiology of multiple sclerosis: a new and extended vascular-ischemic model. *Med Hypotheses*. 1990; 33: 23-9.
2. Brownell B and Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 1962; 25: 315-20.
3. Sun X, Tanaka M, Kondo S, Okamoto K and Hirai S. Clinical significance of reduced cerebral metabolism in multiple sclerosis: a combined PET and MRI study. *Ann Nucl Med*. 1998; 12: 89-94.
4. Bartels K, Grenz A and Eltzschig HK. Hypoxia and inflammation are two sides of the same coin. *Proc Natl Acad Sci U S A*. 2013; 110: 18351-2.
5. Davies AL, Desai RA, Bloomfield PS, et al. Neurological deficits caused by tissue hypoxia in neuroinflammatory disease. *Ann Neurol*. 2013; 74: 815-25.
6. Johnson TW, Wu Y, Nathoo N, Rogers JA, Wee Yong V and Dunn JF. Gray Matter Hypoxia in the Brain of the Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis. *PLoS One*. 2016; 11: e0167196.
7. Marshall O, Chawla S, Lu H, Pape L and Ge Y. Cerebral blood flow modulation insufficiency in brain networks in multiple sclerosis: A hypercapnia MRI study. *J Cereb Blood Flow Metab*. 2016; 36: 2087-95.
8. Inglese M, Adhya S, Johnson G, et al. Perfusion magnetic resonance imaging correlates of neuropsychological impairment in multiple sclerosis. *J Cereb Blood Flow Metab*. 2008; 28: 164-71.
9. Yang R and Dunn JF. Reduced cortical microvascular oxygenation in multiple sclerosis: a blinded, case-controlled study using a novel quantitative near-infrared spectroscopy method. *Sci Rep*. 2015; 5: 16477.
10. Taccone FS, Su F, De Deyne C, et al. Sepsis is associated with altered cerebral microcirculation and tissue hypoxia in experimental peritonitis. *Crit Care Med*. 2014; 42: e114-22.
11. Burrows F, Haley MJ, Scott E, et al. Systemic inflammation affects reperfusion following transient cerebral ischaemia. *Exp Neurol*. 2016; 277: 252-60.
12. Leibowitz SM and Yan J. NF-kappaB Pathways in the Pathogenesis of Multiple Sclerosis and the Therapeutic Implications. *Front Mol Neurosci*. 2016; 9: 84.
13. Haider L, Zrzavy T, Hametner S, et al. The topography of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain*. 2016; 139: 807-15.
14. Martinez Sosa S and Smith KJ. Understanding a role for hypoxia in lesion formation and location in the deep and periventricular white matter in small vessel disease and multiple sclerosis. *Clin Sci (Lond)*. 2017; 131: 2503-24.