Oligodendroglia Are Particularly Vulnerable to Oxidative Damage After Neurotrauma In Vivo

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Journal of Experimental Neuroscience
Volume 12: 1–3

DOI: 10.1177/1179069518810004
SAGE

ABSTRACT: In the paper "Oligodendroglia are particularly vulnerable to oxidative damage after neurotrauma in vivo," we determined the extent of oxidative damage to specific cellular subpopulations and structures within regions vulnerable to secondary degeneration and assessed the effect this had on oligodendroglial function. Comparative assessment of oxidative damage demonstrated selective vulnerability of oligodendroglia, specifically oligodendrocyte progenitor cells (OPCs) to DNA oxidation in vivo. Immunohistochemical fate mapping along the oligodendroglial lineage showed a transient susceptibility of these cells to DNA oxidation, protein nitration, and lipid peroxidation, with mature oligodendrocytes derived immediately after injury more vulnerable to DNA oxidation than their counterparts existing at the time of injury or later derived. In situ hybridization demonstrated a reduction in myelin regulatory factor (MyRF) messenger RNA (mRNA) fluorescence in newly derived mature oligodendrocytes, suggesting a compromise in the production and maintenance of the myelin sheath in these cells. The data imply a deficit in the normal differentiation of OPCs to myelinating oligodendrocytes, associated with a transient increase in oxidative damage, which may contribute to the dysmyelinating phenotype seen at chronic time points after injury. Identifying and understanding the sources of this oxidative damage is integral for the development of therapeutic interventions for neurotrauma.

KEYWORDS: Neurotrauma, myelin, oligodendrocyte, oxidative damage

RECEIVED: October 3, 2018. **ACCEPTED:** October 9, 2018.

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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COMMENT ON: Giacci MK, Bartlett CA, Smith NM, et al. Oligodendroglia are particularly vulnerable to oxidative damage after neurotrauma in vivo. *J Neurosci*. 2018;38:6491-6504. doi:10.1523/JNEUROSCI.1898-17.2018. PubMed PMID: 29915135. https://www.ncbi.nlm.nih.gov/pubmed/29915135

Oligodendroglial Vulnerability to Secondary Degeneration

To our knowledge, there have been no studies directly comparing the vulnerabilities of different cellular subpopulations and structures to oxidative damage following neurotrauma in vivo. Studies comparing the differential vulnerabilities of cellular subpopulations in the central nervous system (CNS) following insult have typically been performed in vitro using primary or mixed cell cultures, and in vivo studies have used percentage cell death as a proxy for damage. However, empirical evidence suggests a particular vulnerability of oligodendrocytes, oligodendrocyte progenitor cells (OPCs), and myelin sheaths to glutamate excitotoxicity.[1] It is thought that the combination of a high metabolic rate with potentially dangerous associated byproducts, high intracellular iron, and low concentrations of antioxidants, specifically, glutathione, renders oligodendrocytes particularly vulnerable to oxidative damage.[2]

Despite this understanding, there have been few studies directly comparing the degree of damage to cellular subpopulations and structures, due to the inherent limitations of traditional immunohistochemistry. The opportunity to explore using NanoSIMS allowed direct comparison between cellular subpopulations and structures in the context of secondary degeneration in vivo for the first time and provided direct evidence that OPCs and oligodendroglia are the most vulnerable, when compared with other cellular subpopulations and structures in the CNS.[3]

Further investigation using immunohistochemical approaches showed that caspase-3 positive, EdU-negative mature oligodendrocytes that were likely pre-existing at the time of injury were associated with a high immunointensity of 8OHdG at 3 days after injury. Caspase-3 cleavage triggers DNA fragmentation, degradation of cytoskeletal and nuclear protein, formation of apoptotic bodies, and expression of ligands recognized by phagocytic cells for uptake, so the data indicate that DNA oxidation leads to cell death.[3] However, the numbers of EdU-negative oligodendrocytes did not decrease until 28 days after injury.[3] It is a possibility that the numbers of these cells were reduced at 14 days after injury; however, this was not assessed.

Delayed loss of oligodendrocytes has been observed following spinal cord injury in the rat, whereby apoptotic oligodendroglial cells were recorded from 6 hours to 3 weeks after injury.[4] Despite the loss of EdU-negative mature oligodendrocytes, the total numbers of mature oligodendrocytes did not decrease, as they were supplemented by EdU-positive oligodendrocytes. The lack of myelin regulatory factor (MyRF) messenger RNA (mRNA) fluorescence in EdU-positive mature oligodendrocytes derived after injury would suggest these cells are surviving with compromised function and are unlikely to contribute to myelination. Additional experiments to ascertain the presence of essential myelin proteins within these newly derived MyRF-deficient oligodendrocytes will allow determination of the biological consequence of decreased MyRF expression.

Source of Oxidative Damage to Oligodendroglia

Most of the observed oxidative damage to oligodendroglia within regions of secondary degeneration occurred 3 days after injury, with markers returning to uninjured levels at 7 and 28 days. Astrocytes, microglia, and infiltrating macrophages are producers of reactive oxygen species (ROS) in neurotrauma and may contribute to the spread of oxidative damage following injury. Astrocytes are complicit in other models of neurotrauma, with astrocytic networks contributing to the spread of oxidative damage from the initial injury site to uninjured adjacent areas via the amplification of Ca^{2+} waves and glutamate excitotoxicity.[5] Hypertrophy of astrocytic immunofluorescence was observed throughout the optic nerve following partial injury, with greater amounts of glial fibrillary acidic protein (GFAP) and nestin seen at 3 days after injury.[6] The increase in astrocytic reactivity coincides with increased oxidative stress in oligodendroglia within regions of secondary degeneration,[3] implicating astrocytic reactivity in mediating oxidative damage to these structures.

Previous research from the Fitzgerald lab has found increased numbers of ED1+ macrophages and Iba1+ microglia in the dorsal injury site in the first 24 hours, followed by a spread to the adjacent surrounding ventral tissue at 3 days,[6] coinciding with increased oxidative damage to oligodendroglia within these regions of secondary degeneration. However, the exact role of the immune response following neurotrauma is unclear. Empirical evidence suggests that microglia do not form stable subsets, and phenotype is determined by a number of competing factors in the tissue, forming complicated, mixed phenotypes with varying levels of activation,[7] therefore probably leading to varying effects on vulnerable oligodendroglial subpopulations.

Immune Responses Following Neurotrauma

We have previously demonstrated a concomitant increase in arg1 and iNOS immunointensity, and co-expression of arg1 and iNOS within individual cells, in nerve vulnerable to secondary degeneration. Immunohistochemical assessment revealed increased iNOS immunoreactivity 7 days after injury and increased arg1 immunoreactivity 7 and 28 days after injury. In addition, the number of CD45-positive cells increased 7 days after injury and CD11b-positive cells increased 7 and 28 days after injury.[8] iNOS produces NO, which acts as a signaling molecule and vasodilator at low concentrations; however, at higher concentrations, it is implicated in immune-mediated cytotoxicity.[9] Arg1 is an inducible enzyme that competes with iNOS for L-arginine and, as a result, is a regulator of iNOS translation in a variety of cells.[10] It has been suggested that arg1 in macrophages promotes Th2 cytokine production, contributing to the resolution of inflammation and tissue repair,[11] supporting the anti-inflammatory function of arg1. The exact role of arg1 in alternatively activated macrophage regulation in the context of neurotrauma remains to be elucidated.

The lack of oxidative damage in oligodendroglia at 7 days after injury suggests that the anti-inflammatory components of arg1 and iNOS preferentially influence the cellular environment. In addition, arg1 immunointensity remains elevated at 28 days after injury, which could be facilitative to an anti-inflammatory environment and potentially account for the reduced oxidative damage in oligodendroglia 7 and 28 days after injury.

Compromised Myelin Generation by Newly Derived Oligodendrocytes

Increased DNA oxidation 3 days after injury in newly derived oligodendrocytes compared with their counterparts existing at the time of injury implies that proliferation influences oligodendroglial vulnerability to oxidative damage.[3] Although the number of mature oligodendrocytes was maintained following injury, MyRF, which is integral in both the development and maintenance of the myelin sheath, is almost completely absent in newly derived oligodendrocytes, in contrast to their previously existing counterparts. Mature oligodendrocytes were identified using CC1; however, expression of CC1 alone does not prove myelin sheath production by these postmitotic cells,[12] and it is a strong possibility that the mature oligodendrocytes derived after injury in this study do not produce myelin sheaths. In vitro knockdown of MyRF in cultured OPCs by RNA interference delayed and reduced the expression of the majority of myelin genes, including myelin basic protein, myelin oligodendrocyte protein, 2′,3′-Cyclic nucleotide 3′ phosphodiesterase, and myelin proteolipid protein, compared with cultured OPCs with no MyRF interference.[12] MyRF knockdown has also led to increased apoptosis in cultured OPCs.[12] Furthermore, conditional knock out of MyRF within the oligodendroglial lineage in vivo leads to severe dysmyelination in the mouse optic nerve as well as increased levels of oligodendroglial cell death.[12]

The dysmyelination phenotype observed at chronic time points after neurotrauma may be due to either a lack of new myelin replacing myelin sheaths structurally compromised by the injury environment or the production of compromised myelin sheaths by newly derived oligodendrocytes. The disruption of myelin genes such as myelin proteolipid protein[13] has been associated with the dysmyelination phenotype which is similarly observed following neurotrauma.

Implications for Human Injury

Animal models of neurotrauma are used to develop an understanding of the cellular and molecular processes driving the progression of injury that cannot be investigated in the clinical setting. The optic nerve is part of the CNS and undergoes the same primary and secondary degenerative processes as other white matter tracts in the CNS, such as those found in the spinal cord. The results from this article suggest that oligodendroglial functioning may be compromised in chronic

neurotrauma lesions, associated with oxidative damage in the acute phase of injury. To our knowledge, there have been no studies investigating the impact of oxidative damage to oligodendrocytes following neurotrauma in humans, making extrapolation from the rodent to human difficult. It is worth noting that there is increased DNA oxidation in oligodendrocyte nuclei of multiple sclerosis (MS) patients.[14] However, these experiments were performed on chronic MS tissue and are not indicative of the acute phase of MS. More work needs to be done to find out how applicable the changes observed in rodents following neurotrauma are to humans.

Final Remarks

The maintenance of oligodendrocytes and their associated myelin sheaths is integral to the proper functioning of neurons. The changes to oligodendrocytes within regions of secondary degeneration are multifaceted and effective treatments are likely to have to address the multiple components of the injury response. Extensive characterization of the immune response and associated increased ROS following neurotrauma will help guide the timing of therapeutic intervention following injury for better functional outcomes. There has already been some success in maintaining OPC numbers using multiple ion channel inhibitors following neurotrauma[15]; however, additional studies assessing the influence of these inhibitors on oxidative damage and differentiation of OPCs are required. It is likely that limiting oxidative damage to oligodendroglia during the acute phase of injury will be associated with improved outcomes in myelin ultrastructure in chronic neurotrauma lesions. In addition to protecting oligodendroglia from oxidative damage, congruent therapies promoting the expression of MyRF in newly derived oligodendrocytes may improve functional outcomes following neurotrauma.

Author Contributions

MG wrote the manuscript; MF edited the manuscript.

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