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Assessment of Axonal and Neuronal Damage in a Model of Excitotoxicity

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Overactivation of ionotropic glutamate receptors plays a central role in the pathophysiology of both gray and white matter injury in a number of neurological conditions such as traumatic spinal cord injury, stroke, multiple sclerosis, Alzheimer's, Parkinson's and Huntington's diseases. Neurons and axons are susceptible to glutamate receptor activation *in vivo*. Among white matter elements, oligodendrocytes are highly vulnerable to excitotoxicity *in vivo* and *in vitro*, mediated mainly through the activation of oligodendroglial AMPA/Kainate receptors. However, data are less clear with regard to glutamate receptor expression in axons. Although some publications report that there are functional glutamate receptors on axons and their activation may play a role in axonal injury, several lines of research have previously suggested that excitotoxic damage to axons is a secondary event mediated by injury to glial cells. Curiously, isolated axons in culture are not susceptible to glutamate toxicity and become vulnerable only when co-cultured with oligodendrocytes, suggesting that excitotoxic injury to axons may involve interaction with oligodendrocytes. Thus, we utilized an *in vivo* model of excitotoxic injury, where AMPA receptor injections to murine spinal cord resulted in the injury to axons, neurons and oligodendrocytes. This model enabled us to study the role of oligodendrocytes in the context of excitotoxic axonal/neuronal injury as well as the potential neuroprotective mechanisms of Dimethyl Fumarate (DMF).

Hence, we made use of transgenic mice that express diphtheria toxin (DT) receptors under the control of the MOG promoter (MOGi-Cre/iDTR) and backcrossed these with *thy1*-YFP-H mice expressing yellow fluorescent protein (YFP) under control of the thy1 promoter. Administration of DT in these mice resulted in the ablation of oligodendrocytes in a dose-, time- and administration route-dependent way. Treatment with diphtheria toxin (0.4 µg/ml-1.1 µl) via intraspinal injections resulted in the local ablation of mature oligodendrocytes and a slight demyelination in 9 days without generating any axonal injury. After 9 days we injected a glutamate receptor agonist, AMPA (30 mM, 0.07µl) into lumbar dorsal columns in DT- and vehicle-treated MOGi-Cre/iDTR-*thy1*-YFP-H mice. Axonal and neuronal

morphology were examined in both groups after 24 hours. Our results suggest that injection of AMPA into the lumbar dorsal columns induced similar axonal and neuronal degeneration in oligodendrocyte-depleted and the control mice. In our *in vivo* model, axons are damaged by excitotoxicity independent of presence or absence of oligodendrocytes. This data suggests that different mechanisms may apply to excitotoxic axonal damage *in vitro* and *in vivo*, e.g. axons in vivo but not in vitro may be directly susceptible to excitotoxicity.

Moreover, possible neuroprotective effects of DMF and its mechanisms were investigated utilizing the *in vivo* excitotoxicity model through intraspinal AMPA microinjections. Initially, DMF (200 mg/kg) or vehicle was orally administrated in C57BL/6 mice for 3 and 14 days. The HO-1 mRNA levels in CNS were detected via RT-PCR and HO-1 protein expression in cervical spinal cord was determined by immunohistochemistry. Subsequently, mice were pre-treated orally and intraperitoneally with DMF (200mg/kg) or vehicle for 3 days followed by AMPA injection into the spinal cord with different AMPA doses. Our results suggest that HO-1 mRNA and protein levels has shown a tendency towards increase as a result of oral DMF administration. Moreover, intraperitoneal DMF pre-treatment for 3 days resulted in a significantly decreased neuronal damage and improved behavioral outcomes as compared to the vehicle-treated mice after AMPA injections, indicating a possible neuroprotective effect of DMF. This finding might provide a rationale to test DMF (Tecfidera) in progressive multiple sclerosis where neurodegeneration is believed to play an importnat role.

Elucidating the mechanisms for glutamate-mediated axonal and neuronal injury may reveal new targets for neuroprotective therapy.