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GLIAL CELLS EXPRESS NUCLEAR NRF2 AFTER FUMARATE TREATMENT FOR MULTIPLE SCLEROSIS AND PSORIASIS

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BG12 (Tecfidera) is an oral medication composed of dimethyl fumarate (DMF) that has been approved for the treatment of relapsing-remitting multiple sclerosis (MS). DMF has anti-inflammatory but also putative neuroprotective mechanisms of action. Neuroprotection is hypothesized to be mediated by activation of the nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) antioxidant pathway. Nrf2 nuclear translocation was shown in neurons, oligodendrocytes, and astrocytes in experimental autoimmune encephalomyelitis, an animal model of MS, as well as in astrocytes in vitro after DMF application.^{1,2} This nuclear translocation of the transcription factor Nrf2 promoted neuronal and astrocytic survival after oxidative stress in vitro.^{1,2} Oxidative stress is an important component leading to tissue destruction and neurodegeneration in MS pathology.³ To some degree Nrf2 is also induced as a natural response in MS brains, as has been shown in autopsy studies.¹

Thus far there is no proof of DMF-induced Nrf2 expression in situ in MS tissue. In the present study, we analyzed a biopsy specimen from a 33-year-old woman with relapsing-remitting MS who underwent more than 1 year of DMF treatment at a dosage of 240 mg twice a day. When the patient deteriorated, showing a severe right-sided homonymous hemianopsia, an MRI was performed, revealing a large enhancing lesion in the contralateral occipital lobe (figure, A–C). To exclude a possible CNS lymphoma unfolding during novel MS immunotherapy, a brain biopsy was performed 8 weeks after the last DMF dosage.

Histology showed an inactive inflammatory demyelinated MS lesion (figure, D–F). The patient stabilized after biopsy, showing only residual hemianopsia.

We identified the number of all Nrf2-positive nuclei irrespective of the cell type in our patient treated with DMF (figure, G) and compared the results with inactive demyelinated MS lesions from 6 patients not treated with DMF (figure, H). Higher numbers of Nrf2-positive nuclei were present in the patient treated with DMF (460/mm²) than in the control patients

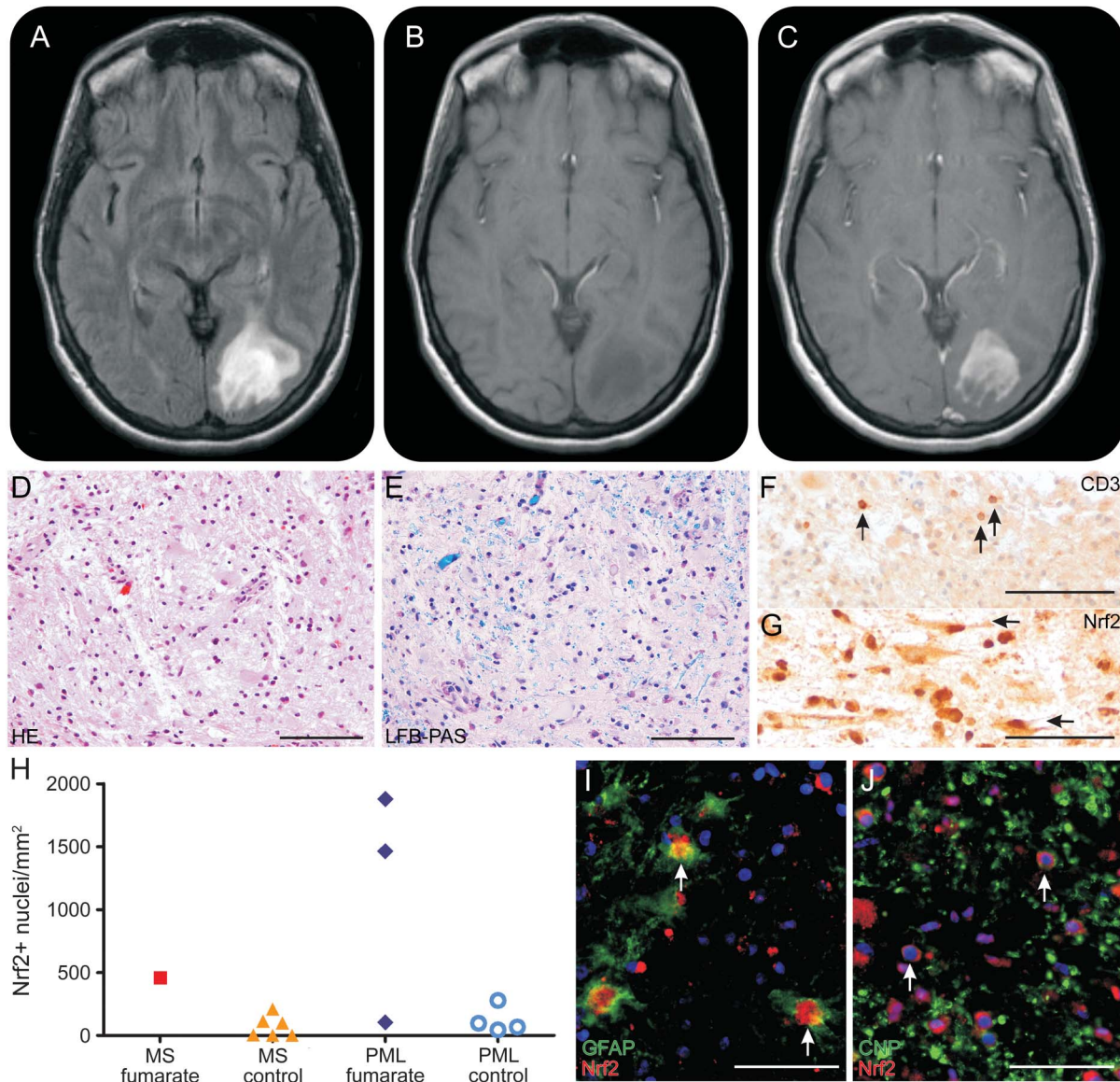
with MS. The number of Nrf2-positive nuclei was more than 6-fold higher after DMF treatment (figure, H). We then performed double immunohistochemical stainings to identify Nrf2-positive cell populations. The strongest Nrf2 signal was found within astrocytes that showed nuclear and cytoplasmic Nrf2 expression (figure, I). Most oligodendrocytes presented with a cytoplasmic Nrf2 signal (figure, J), although single oligodendrocytes had a nuclear Nrf2 signal as well (not shown). Some macrophages/microglial cells showed a weak nuclear and cytoplasmic Nrf2 expression, whereas lymphocytes had a cytoplasmic Nrf2 expression only (not shown). Gray matter was not available for analysis.

Fumarates are a common therapy for the Th1-mediated skin disease psoriasis. The long-established medication, Fumaderm, is composed of 120 mg DMF and 95 mg ethylhydrogenfumarate. Single cases of progressive multifocal leukoencephalopathy (PML) in connection with Fumaderm treatment have been reported. We were able to enumerate Nrf2-positive nuclei in 3 PML lesions from patients with ongoing fumarate treatment (figure, H). Two patients showed higher numbers of Nrf2-positive nuclei (1,878/mm² and 1,464/mm²), and the last patient presented with similar numbers (104/mm²) as PML controls (mean of 4 lesions: 122 Nrf2-positive nuclei/mm²). Again, astrocytes showed the strongest Nrf2 signal.

In conclusion, the present results suggest that Nrf2 is translocated into nuclei after fumarate treatment. This may indicate that fumarates act by activating antioxidative pathways, as suggested by animal and cell culture experiments. Although our study is limited by the analysis of only 1 patient with MS and 3 patients with psoriasis undergoing fumarate treatment, the strong increase in the number of Nrf2-positive nuclei supports this hypothesis. It remains unclear why 1 patient with psoriasis did not show an increase in Nrf2-positive nuclei; this could be due to noncompliance in taking the medication. Caution is advised when interpreting our findings since “natural inflammation” in MS or PML may also cause an increase in Nrf2-expressing nuclei, reflecting a physiologic response.

We found nuclear Nrf2 expression predominantly in astrocytes. Prior studies in neurodegenerative

Figure Nuclear Nrf2 expression after fumarate therapy



A new left occipital fluid-attenuated inversion recovery hyperintense (A), T1 hypointense (B), and gadolinium-enhancing lesion (C, T1 + gadolinium) under fumarate therapy for relapsing-remitting multiple sclerosis (MS) led to biopsy. The biopsy specimen shows an inactive demyelinated white matter lesion with macrophages and reactive astrocytes (D, hematoxylin & eosin). The lesion is demyelinated (E, Luxol fast blue/periodic acid-Schiff staining) without any signs of active myelin degradation. Only scant lymphocytic infiltrate is present (F, anti-CD3 staining, lymphocytes indicated by arrows). Numerous cells show nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) expression (G, anti-Nrf2 staining, Nrf2+ astrocytes indicated by arrows). Within this inflammatory demyelinated MS lesion, the Nrf2-positive nuclei were determined. Six-fold higher numbers of Nrf2-positive nuclei were found compared to MS controls (H). The strongest nuclear Nrf2 signals were present in astrocytes (I), whereas oligodendrocytes showed mostly a cytoplasmic Nrf2 staining (J). Furthermore, patients with psoriasis treated with fumarates showed higher numbers of Nrf2-positive nuclei than progressive multifocal leukoencephalopathy (PML) controls (H). Scale bars: D-G: 100 μ m, I and J: 50 μ m.

models such as Parkinson disease showed that effects via astrocytes may be critical for Nrf2-mediated neuroprotection.^{4,5} Downstream mechanisms of Nrf2 activation and nuclear translocation may include increased glutathione (GSH) levels in astrocytes.⁶ GSH represents the main cellular antioxidant against reactive oxygen species, thus defending oxidative stress. The effects of DMF are manifold and also include immunomodulatory and immunosuppressive effects, for example, the reduction of peripheral T

lymphocytes.⁶ Other pathways such as the nuclear factor- κ B activation are affected by DMF treatment.⁶ However, our results showing a nuclear translocation of Nrf2 after fumarate treatment may suggest that this neuroprotective pathway is important for mediating the therapeutic effects of fumarates.

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Author contributions: Dr. Metz designed the study, acquired the data, analyzed and interpreted the data, and drafted and revised the manuscript. Sarah Traffehn acquired the data, interpreted the data, and critically revised the manuscript. Dr. Straßburger-Krogias, Dr. Keyvani, Dr. Bergmann, Dr. Nolte, Dr. Weber, and Dr. Bartsch interpreted the data critically and revised the manuscript. Dr. Gold and Dr. Brück designed the study, analyzed and interpreted the data, and critically revised the manuscript.

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