Orally Administered Diroximel Fumarate Induces Activation of the Nuclear Factor (Erythroid-Derived 2)–Like 2 Transcriptional Pathway

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OBJECTIVE

- To measure MMF exposure and Nrf2-dependent transcriptional pharmacodynamic responses in C57BL/6 mice after orally administered DRF. Orally administered DMF served as a positive control.

CONCLUSIONS

- MMF exposure levels in blood plasma and tissue were similar after a single oral administration of DRF or DMF in C57BL/6 mice.
- The MMF-mediated Nrf2 activation profiles of the diester fumarates DRF and DMF are very similar, suggesting each may confer comparable neuroprotective, cytoprotective, and immunomodulatory effects.

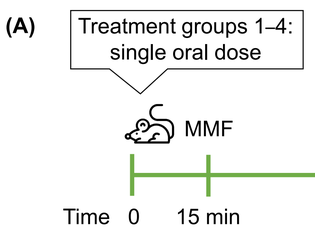
Introduction

- Diroximel fumarate (DRF) is a novel oral fumarate indicated in the United States for patients with relapsing forms of multiple sclerosis (MS).[1]
- DRF is rapidly converted to monomethyl fumarate (MMF), the same active metabolite of dimethyl fumarate (DMF),[2] and is expected to have a similar pharmacodynamic effect as DMF.
  - DRF is differentiated from DMF by its improved gastrointestinal tolerability, demonstrated in a head-to-head clinical study versus DMF.[3]
- Diester fumarates such as DRF and DMF are hypothesized to impact MS pathophysiology via nuclear factor (erythroid-derived 2)–like 2 (Nrf2) dependent mechanisms of neuroprotection and cytoprotection, in addition to immunomodulatory effects.[4,5]
  - The Nrf2 transcriptional pathway induces expression of a number of antioxidant response genes, including aldo-keto reductase family 1, member b8 (*AKR1B8*), heme oxygenase 1 (*HMOX1*), and oxidative stress-induced growth inhibitor 1 (*OSGIN1*).[5]

Methods

- C57BL/6 mice were administered a single oral dose of DRF 192.5 mg/kg (n = 18), DRF vehicle (n = 18), DMF 100 mg/kg (n = 18), or DMF vehicle (n = 18; Figure 1).
  - DMF was included as a positive control group.
- Mice were euthanized at 15 minutes, 2 hours, or 6 hours after dose (6 per timepoint in each group); whole blood (for plasma preparation) and tissue samples (brain, jejunum, kidney, and spleen) were collected.
- MMF exposure levels were evaluated at 15 minutes (plasma, brain, jejunum, kidney, and spleen), 2 hours (plasma), and 6 hours (plasma) after dosing using liquid chromatography tandem mass spectrometry.
- Pharmacodynamic transcriptional responses assessing Nrf2 activation were analyzed at 2 hours and 6 hours after dosing in brain, kidney, jejunum, and spleen.
  - RNA from brain, jejunum, kidney, and spleen was extracted using RNeasy 96 Universal Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol.
  - RNA samples were reverse transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA) and analyzed by quantitative real-time polymerase chain reaction, using primers specific for *AKR1B8*, *HMOX1*, and *OSGIN1* (6-FAM™ dye-labeled TaqMan® MGB™ probes; Thermo Fisher Scientific).

Figure 1. (A) Study Design, **(B)** Dosing, and **(C)** Tissue Collection Timepoints in DRF- and DMF-Treated C57BL/6 Mice



(B)

Treatment Groups[a] (n =18 per group; 6 per timepoint)
Group 1: DRF 192.5 mg/kg
Group 2: DRF vehicle
Group 3: DMF 100 mg/kg
Group 4: DMF vehicle

(C)

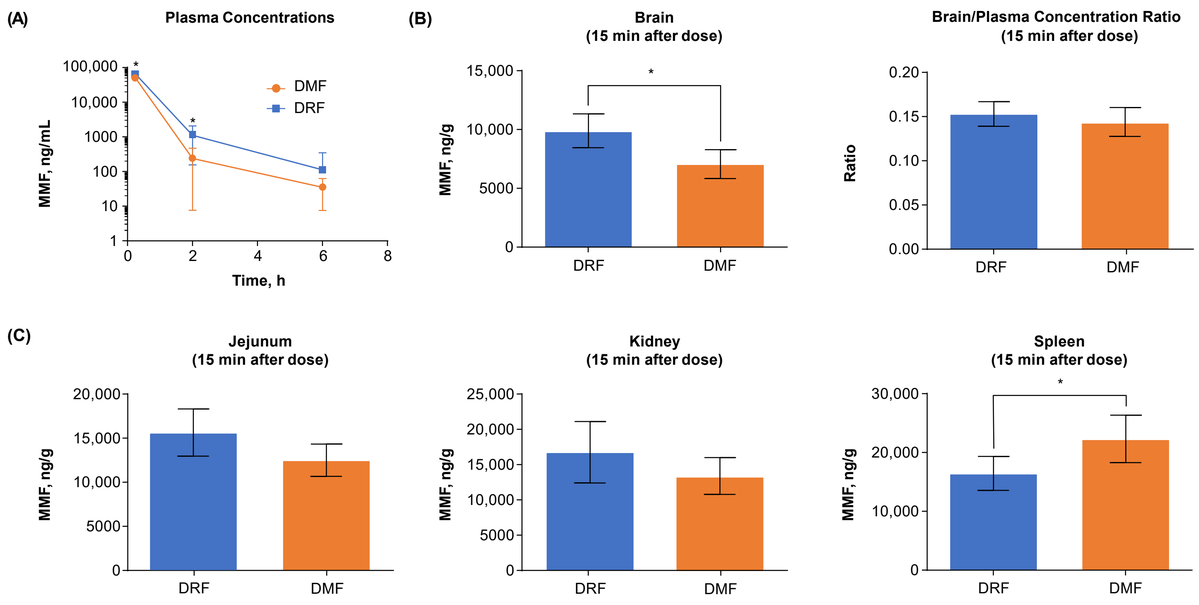
Time of Collection	MMF Exposure	Pharmacodynamics/ Nrf2 Activation (qRT-PCR)
15 min	Plasma, brain, jejunum, kidney, spleen	NA
2 h	Plasma	Brain, jejunum, kidney, spleen
6 h	Plasma	Brain, jejunum, kidney, spleen

DMF = dimethyl fumarate; DRF = diroximel fumarate; MMF = monomethyl fumarate; NA = not applicable; Nrf2 = nuclear factor (erythroid-derived 2)–like 2; qRT-PCR = quantitative real-time polymerase chain reaction
[a]Treatments administered as a single oral dose.

Results

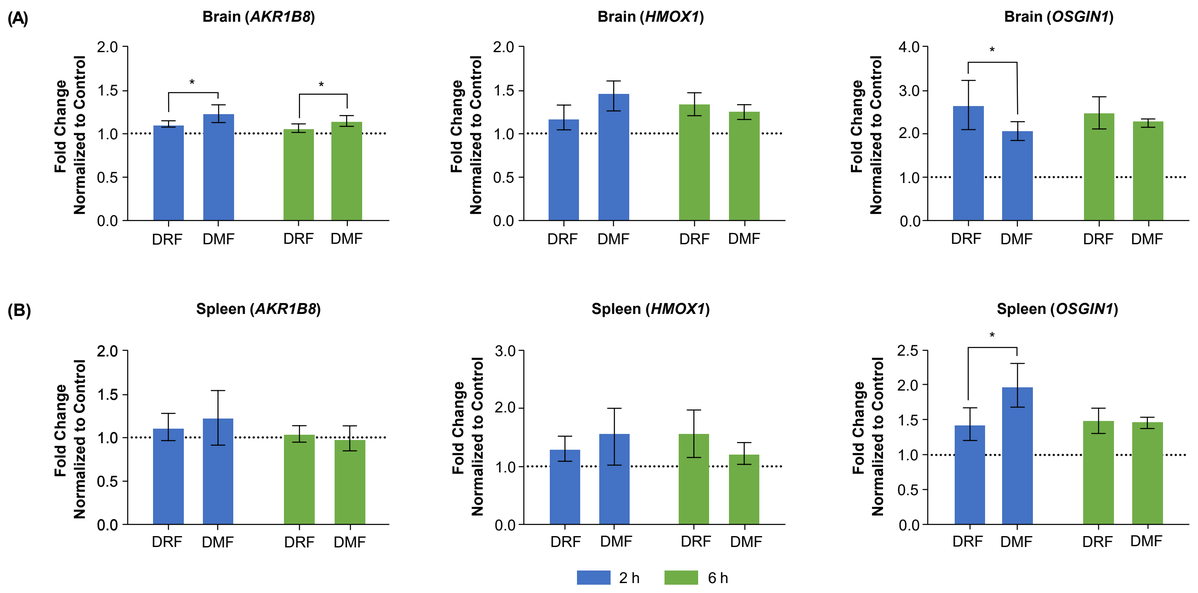
- At 15 minutes after dose, MMF exposure with DRF and DMF was comparable in plasma and tissues, with slight differences observed in brain and spleen (Figure 2).
  - MMF concentrations were higher after DRF administration in plasma (15 minutes and 2 hours after dose) and brain (15 minutes after dose), but there was no difference in the brain to plasma ratios of MMF (Figure 2A, B).
  - MMF concentrations were higher after DMF administration in spleen at 15 minutes after dose (Figure 2C).
- After oral administration of DRF, expression of *AKR1B8*, *HMOX1*, and *OSGIN1* genes were elevated in the brain, jejunum, kidney, and spleen at 2 and 6 hours after dose compared with vehicle-treated animals (≥ 1-fold change vs. vehicle control; Figures 3 and 4).
- At 2 hours and 6 hours after dose, expression levels of *AKR1B8*, *HMOX1*, and *OSGIN1* were overall similar between DRF- and DMF-treated animals (Figures 3 and 4).

Figure 2. Pharmacokinetic Profiles of MMF After a Single Oral Dose of DRF or DMF in C57BL/6 Mice



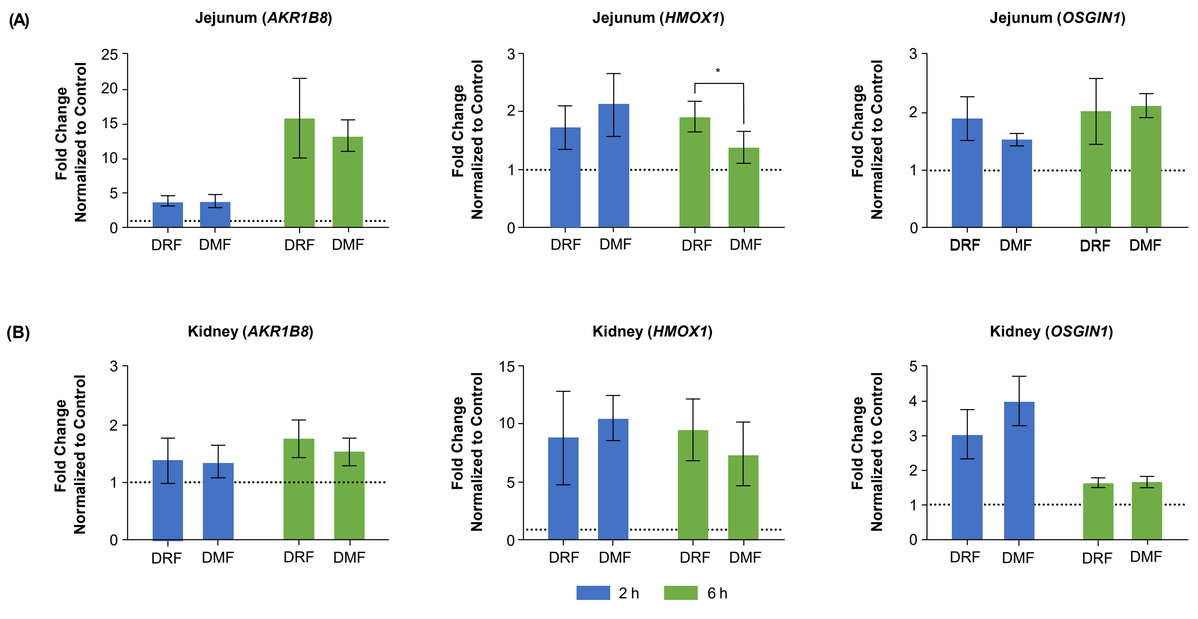
DMF = dimethyl fumarate; DRF = diroximel fumarate; MMF = monomethyl fumarate
(A) Plasma exposure of MMF after oral administration of DRF or DMF. **(B)** Brain exposure of MMF 15 minutes after oral administration of DRF or DMF and brain to plasma concentration ratios after oral administration of DRF or DMF. **(C)** Jejunum, kidney, and spleen exposure of MMF 15 minutes after oral administration of DRF or DMF. Columns/data points represent the mean of 6 animals, and error bars are the SD of the mean. Statistical comparisons were performed using the Mann-Whitney test, where significant differences were defined by a p value < 0.05 (*).

Figure 3. Pharmacodynamic Responses in **(A)** Brain and **(B)** Spleen Following a Single Oral Dose of DRF or DMF in C57BL/6 Mice



DMF = dimethyl fumarate; DRF = diroximel fumarate
Transcriptional responses in the **(A)** brain and **(B)** spleen of *AKR1B8*, *HMOX1*, and *OSGIN1* at 2 hours and 6 hours after oral administration DRF or DMF. Columns represent the mean of 6 animals, and error bars are SD of the mean. Statistical comparisons were performed using the Mann-Whitney test, where significant differences were defined by a p value < 0.05 (*).

Figure 4. Pharmacodynamic Responses in **(A)** Jejunum and **(B)** Kidney Following a Single Oral Dose of DRF or DMF in C57BL/6 Mice



DMF = dimethyl fumarate; DRF = diroximel fumarate
Transcriptional responses in the **(A)** jejunum and **(B)** kidney of *AKR1B8*, *HMOX1*, and *OSGIN1* at 2 hours and 6 hours after oral administration DRF or DMF. Columns represent the mean of 6 animals, and error bars are SD of the mean. Statistical comparisons were performed using the Mann-Whitney test, where significant differences were defined by a p value < 0.05 (*).

References 1. Vumerity [prescribing information]. Cambridge, MA: Biogen; 2019. 2. Tecfidera [prescribing information]. Cambridge, MA: Biogen; 2017. 3. Naismith RT, et al. *CNS Drugs*. 2020;34(2):185-196. 4. Linker RA, et al. *Brain*. 2011;134(pt 3):678-692. 5. Brennan MS, et al. *Antioxid Redox Signal*. 2016;24(18):1058-1071. Disclosures MJP, AMT, DG, KK, JM, and EC-M: employees of and hold stock/stock options in Biogen. Acknowledgments This study was sponsored by Biogen (Cambridge, MA, USA). Writing and editorial support for the preparation of this poster was provided by Excel Scientific Solutions (Fairfield, CT, USA): funding was provided by Biogen.