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Chapter

Repurposing Fumaric Acid Esters to Treat Conditions of Oxidative Stress and Inflammation: A Promising Emerging Approach with Broad Potential

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Abstract

The medicinal benefit of salts of fumaric acid and its esters (FAE), known as fumarates (mono and dimethyl fumarate), was realized many years ago. Early on, FAE were derived from plants and mushrooms (e.g., *Fumaria officinalis, Boletus fomentarius var. pseudo-igniarius*). The FAE containing formulation Fumaderm® was licensed in Germany for the treatment of psoriasis in 1994. Recently, a clinical formulation of dimethyl fumarate known as BG12 (Tecfidera) was approved for use in the United States, New Zealand, Australia, European Union, Switzerland, and Canada for the treatment of multiple sclerosis. Others and we have assessed the potential benefit of FAE in a number of disease conditions that are diverse with respect to etiology but unified with regard to the involvement of inflammation and oxidative stress. Hence, a FAE-based drug with robust anti-oxidative and anti-inflammatory effects that is already US-FDA approved is a perfect contender for repurposing and rapid clinical implementation for their management. There is a burgeoning literature on the use of FAE in the prevention and treatment of diseases, other than psoriasis and MS, in which oxidative stress and/or inflammation are prominent. This chapter highlights critical information gleaned from these studies, exposes lacunae of potential importance, and provides related perspectives.

Keywords: fumaric acid esters, oxidative stress, Nrf2, antioxidant

1. Introduction

The salts of fumaric acid (known as fumarates) occur naturally in some plants (*Fumaria officinalis*) and mushrooms. Traditionally, aerial parts of *Fumaria officinalis* (common fumitory, drug fumitory or earth smoke) have been utilized for the treatment of various skin diseases [1]. Because of its utilization as an herbal remedy for skin ailments, German chemist W. Schweckendiek, who was suffering

from psoriasis, developed an interest in and isolated fumaric acid esters (FAE) from the plant extract. Excited about the positive effects of the FAE mixture on his own psoriatic lesions, he began offering it also to other psoriasis patients. Schweckendiek later published his findings on the beneficial effects of FAE in psoriasis [2], effects that he believed to be attributed to the improvements of this fumarate therapy on dysregulation of the citric acid cycle, the potential underlying cause of psoriasis. Nonetheless, with advancements in the understanding of psoriasis, his hypothesis was found to be incorrect. However, his preliminary observations laid the foundation for the successful development of a drug to treat psoriasis and interestingly, multiple sclerosis.

In 1994, some three decades following Schweckendiek's initial report, a fumaric acid mixture composed in large (60%) of dimethyl fumarate (DMF) and ethyl hydrogen fumarates was authorized for the treatment of psoriasis in Germany under the brand name Fumaderm® [3]. In the clinical setting, Fumaderm® proved effective against moderate to severe forms of psoriasis. To date, it remains to be the most widely used oral compound for psoriasis therapy in Germany. However, Fumaderm® was not licensed and currently remains unlicensed for use in the UK and US [4]. Despite this, results establishing DMF to be the major active principle in the Fumaderm® led to numerous clinical and experimental studies worldwide on the immunomodulatory potential of Fumaderm® and DMF in other immune-mediated diseases [5, 6]. The extremely positive results that emanated from these studies led to DMF being tested clinically for the treatment of relapsing-remitting multiple sclerosis (RRMS). Like the original discovery of FAEs, the exploratory clinical trial of FAE for MS was performed in Germany [7]. In this trial, Fumaderm® was given to 10 patients with highly active RRMS; six patients completed the 70-week trial. Magnetic resonance imaging (MRI)-based results showed that Fumaderm® significantly reduced the number of gadolinium-enhanced lesions as well as lesion volumes without further worsening of any clinical parameters [7]. Although the overall safety profile of Fumaderm® was found to be favorable in this study, the associated unwanted gastrointestinal discomforts were a major concern. Although this initial study was a small, single-center, MRI-based and open-label clinical trial, it set the stage for a number of subsequent MS trials with DMF.

After Fumaderm® was licensed to be used in Germany, efforts to develop an improved formulation with better tolerability began. This culminated ultimately in the introduction of BG12 (brand name Tecfidera) a modified FAE formulation [8–10]. Indeed BG-12, comprised only of DMF made available in enteric-coated micro tablets, showed better gastrointestinal tolerability compared to Fumaderm® and following several clinical trials, this gastro-resistant, delayed-release formulation of DMF was ultimately approved for use in the United States, New Zealand and Australia for the treatment of relapsing forms and relapsing MS, respectively, and in the European Union, Switzerland and Canada for the treatment of RRMS [11]. A plethora of additional information exists on the use of DMF in the treatment of MS and psoriasis. For further reading on DMF and MS, please refer to the following referenced excellent reviews [12–17].

Drug repurposing is a highly appreciated strategy in the pharmaceutical industry [18]. The fact that agents have been previously tested prior testing of in humans and therefore a wealth of detailed information is already available regarding pharmacology, formulation and safety profile is a huge advantage! Such new candidate therapies can often be fast-tracked for clinical trials and related approval by the U.S. Food and Drug Administration. There is a burgeoning literature on the use of FAE in the prevention and treatment of diseases, other than psoriasis and MS,

in which oxidative stress and/or inflammation are prominent. The present review highlights critical information gleaned from these studies and exposes and provides perspectives on lacunae of potential importance.

2. Pharmacokinetics of fumaric acid esters

Dimethyl fumarate (PubChem CID: 637568), described as a "white crystalline compound with a fruit-like taste" [19], is a dimethyl ester of fumaric acid with the official chemical name of trans-1,2-ethylene carboxylic acid dimethyl ester [20]. Because of its rapid degradation by intestinal esterase, DMF does not cross the intestinal wall in significant amounts [21]. Thus, because of its short-lived activity, evidence of direct, sustained anti-inflammatory or antioxidant effects derived directly from DMF is limited [22]. Instead, monomethyl fumarate (MMF; PubChem CID: 21721168), the product of DMF metabolism by intestinal esterase, is said to be the main active metabolite [23]. This is confirmed by pharmacokinetic studies that demonstrate following oral DMF intake, serum concentrations of MMF peak within 2–2.5 h and its half-life is approximately 1 h [24]. Further, the ingestion of DMF along with a high fat/high-calorie diet was found to interfere with intestinal absorption, delaying the systemic peak of MMF significantly [16, 17]. Following doses of delayed-release DMF of up to 240 mg, the mean C_{max} of MMF in healthy human subjects was 1.43 μg/ml with a corresponding MMF area under the curve of 2.41 μg h/ml. There was no evidence of accumulation after multiple doses (e.g. 240 mg delayed-release DMF three times daily for 2 days) as MMF concentrations fell below detectable limits at the end of day 1 and day 2 [24]. MMF is eliminated primarily through breathing; negligible amounts of intact MMF are excreted through urine or feces. Additionally, there is no evidence of cytochrome P450-dependent metabolism of the compound in the liver [25]. Because of the lack of cytochrome P 450 involvement, DMF has very limited drug–drug interactions. Congruent with the above, both DMF and MMF have been popularly used for various pre-clinical pharmacological studies aimed at the testing and development of new therapeutics for various indications. The intestinal metabolism of DMF and diroximel fumarate (DRF), two current clinical FAE formulations is shown in **Figure 1**.

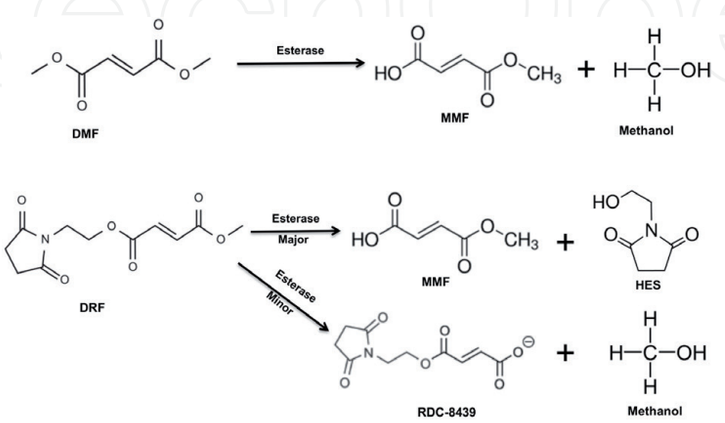

Figure 1.
Metabolism of fumaric acid esters. Clinical formulations of FAE are composed of dimethyl fumarate (DMF) or diroximel fumarate (DRF). Following oral administration, intestinal esterase metabolizes both DMF and DRF into the major bioactive ingredient MMF (monomethyl fumarate). Methanol, hydroxyethyl succinimide (HES) and RDC-8439 are also produced but only as minor metabolites (< 10%).

3. Mechanism of action: fumaric acid esters

Despite the numerous *in vitro* and *in vivo* studies that have been conducted over the years, the mechanism of action of FAE is still not fully understood and novel aspects continue to emerge. The generic hypothesis to explain the benefits of FAE is that DMF/MMF interferes with the cellular redox system by inducing a strong antioxidant response. Indeed, the robust induction of Nrf2 (nuclear factor E2 (erythroid-derived 2)-related factor) by DMF/MMF has been well described (**Figure 2**). In cells, DMF/MMF leads to the nuclear translocation of Nrf2, a phenomenon that is known to in turn, enhance the expression of antioxidant enzymes [26]. Specifically, it has been shown that MMF induces alkylation of a critical reactive thiol, Cys151, on Keap1 (Kelch-like ECH associated protein 1) which results in the release of Nrf2 [26, 27]. Once dissociated from Keap-1, Nrf2 translocates to the

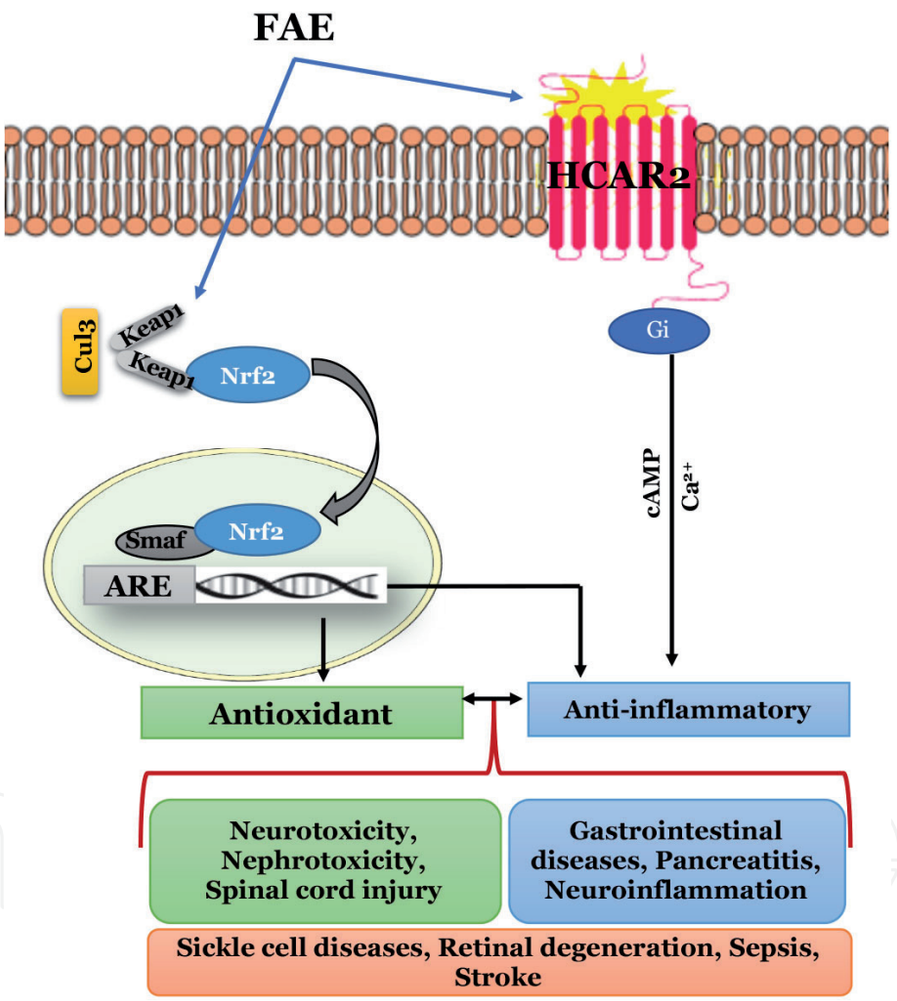

Figure 2.
Involvement of Nrf2-dependent and independent mechanisms in FAE-mediated antioxidant and anti-inflammatory effects. Fumaric acid esters (DMF/MMF) disrupt Keap1-Nrf2 binding to induce nuclear translocation of Nrf2 which in turn, activates a number of downstream antioxidant response genes. This mode of action of FAE is well known and is purported to be responsible for the positive actions of FAE in neurotoxicity, nephrotoxicity, and spinal cord injury. Additionally, however, MMF, the major bioactive ingredient of FAE, is an agonist of HCAR2, a G_i-protein coupled membrane receptor that potentiates robust anti-inflammatory signaling. Various studies have shown that while FAE-mediated Nrf2 signaling elicits both antioxidant and anti-inflammatory responses, HCAR2-dependent signaling predominantly provides an anti-inflammatory effect. The HCAR2-mediated actions of FAE have been implicated its protective effects in gastrointestinal diseases, pancreatitis and neuroinflammation. Importantly however, the combined actions (Nrf2- and HCAR2-mediated) have been demonstrated in several pathologic conditions (sickle cell disease, retinal degeneration, sepsis and stroke). FAE, fumaric acid esters; HCAR2 or HCA2, hydroxycarboxylic acid receptor 2; DMF, dimethyl fumarate; MMF, monomethyl fumarate; Keap1; kelch-like ECH associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; ARE; antioxidant responsive element.

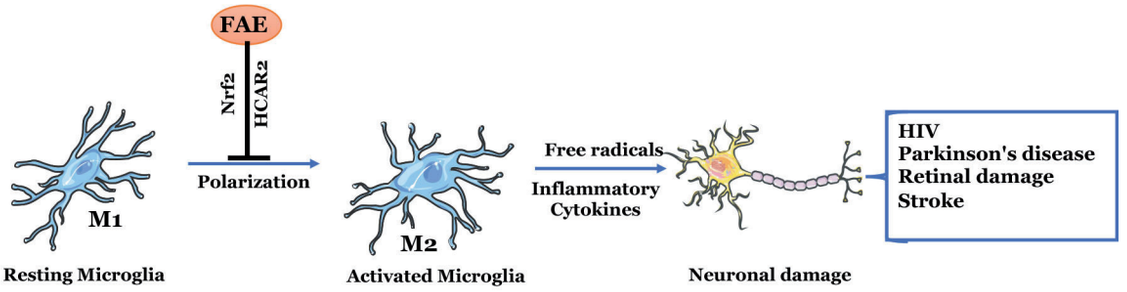

Figure 3.
The role of FAE in regulating microglia activation. FAE (DMF/MMF) are known to induce Nrf2 and to activate HCAR2. Various studies have shown that via these mechanisms, FAE prevent the polarization of microglia from the M1 (resting) phenotype to the M2 (active and thereby pro-inflammatory) phenotype, the consequences of which are reduced free radical and pro-inflammatory cytokine production. This action of FAE is thought to underlie the neuroprotective effects of the drug in conditions like HIV-induced neuroinflammation, Parkinson's disease, retinal degeneration, and stroke. FAE, fumaric acid esters, HCAR2, hydroxycarboxylic acid receptor 2; DMF, dimethyl fumarate; MMF, monomethyl fumarate; Nrf2, nuclear factor erythroid 2-related factor 2; HIV, human immunodeficiency virus.

nucleus and therein, binds to the antioxidant response element (ARE) of an array of antioxidant target genes thereby upregulating their expression and related activity. This effect was corroborated in Nrf2-deficient cells in which the antioxidant effects of DMF/MMF were lost [27].

The majority of preclinical studies of DMF/MMF, highlight the Nrf2-mediated mechanism of the drug as the principal factor underlying its therapeutic effects. However, DMF/MMF has also been shown to elicit a robust anti-inflammatory response. This additional desirable effect is thought to be accomplished via the inhibition of NF-kB translocation into the nucleus, an action that impacts negatively the expression of a plethora of inflammatory cytokine, chemokine, and adhesion molecule genes. Relevant also to the anti-inflammatory effects of DMF/MMF, is hydroxycarboxylic acid receptor 2 (HCAR2; GPR109A)-dependent signaling (**Figure 2**). MMF is a strong agonist of HCAR2. DMF activates the receptor as well although with a comparably lower affinity [28]. In a study by Chen et al. 2014 it was shown that DMF treatment reduced pathological features of experimental autoimmune encephalomyelitis in WT mice, but not in *Hcar2*$^{-/-}$ mice, indicating the importance of HCAR2-mediated signaling by DMF [29]. In another study, Parodi et al. [30] demonstrated the importance of HCAR2 to the anti-inflammatory effects of MMF in microglia. Specifically, it was reported that MMF could modulate microglia activation through inhibition of the NF-κB pathway via the AMPK/SIRT-1 axis. MMF treatment to microglia cells resulted in the activation of the HCAR2 receptor via enhanced intracellular calcium levels, an effect that prevents microglial polarization into an inflammatory phenotype (**Figure 3**). Downstream, it induced CAMKK (Calcium/calmodulin-dependent protein kinase kinase 2) dependent activation of AMPK/SIRT-1 axis which also contributes to reduced inflammation. Several other studies have also reported on the HCAR2 receptor-dependent and independent anti-inflammatory effects of FAE in additional cell types including keratinocytes [31–33] and epithelial cells of the retina [34, 35].

4. Role of FAE in inflammatory and oxidative stress conditions

Herein we highlight the findings of preclinical studies on the use of DMF/MMF to counter inflammation and oxidative stress associated with the pathogenesis of pathological conditions other than psoriasis and MS (**Figure 2**). A summary is provided in **Table 1**.

Disease condition	Experimental model	Effective dose	Outcomes	References
Cerebral ischemia–	Middle cerebral artery occlusion in rats	25 and 50 mg/kg DMF (i.g.)	DMF protected against experimental stroke by inducing immunomodulatory and antioxidant response	[36]
	Middle cerebral artery occlusion in mice	30 and 45 mg/kg DMF and MMF (i.p.)	DMF and MMF suppressed glial activation via increasing the expression of Nrf2	[37]
Experimental colitis	a. Mice treated with DNBS. b. IL-10$^{-/-}$ mice.	30 and 100 mg/kg DMF (i.g.)	DMF induced antioxidant response by regulating SOD-2 and inflammation by Nf-kB signaling to reduce colitis.	[38]
	Mice treated with 3% (w/v) DSS drinking water	30 and 60 mg/kg DMF (i.g.)	DMF alleviated DSS-induced colitis by regulating Nrf2-mediated inhibition of NLRP3 inflammasome	[39]
Intracerebral hemorrhage	Intra-striatal injection of autologous blood in rats and mice	15 mg/kg DMF (i.g.)	DMF can ameliorate ICH-mediated injury with a therapeutic window of at least 24 h	[40]
	Mice using either the collagenase injection model (cICH) or the autologous blood (bICH)	100 mg/kg (i.p.)	DMF-induced dissociation of Nrf2 from Keap1, and the consequent casein kinase 2 phosphorylation of Nrf2, resulted in neuroprotection after ICH	[41]
Nephrotoxicity	Rats treated with 20 mg/kg Cyclosporin A for 28 days	50 mg / kg DMF (i.g.)	DMF reduced nephrotoxicity by inhibiting oxidative stress and inflammation	[42]
Neurotoxicity	Mice treated with 10 nmol sodium nitroprusside	60 and 200 mg/kg DMF (i.g.)	DMF reduced neurotoxicity by activating HO-1.	[43]
Pancreatitis	Rats treated with 2.5 g/kg L-arginine	25 mg/kg DMF (i.g.)	DMF was effective in ameliorating the histological lesions and biochemical abnormalities and improving beta-cell function	[44, 45]
	Rats treated with 3 g/Kg L-arginine	25 mg/Kg DMF (i.g.)	DMF treated rats showed reductions in the severity of inflammatory cell infiltration, acinar damage, perilobar edema, and cell necrosis	[46]
Parkinson's disease	6-OHDA-induced neurotoxicity in mice	50 mg/kg DMF (i.g.)	DMF reduced neurotoxicity by Nrf2 mediated antioxidant response.	[47]
	Mice treated with MPTP	100 mg/kg MMF/DMF (i.g.)	DMF and MMF exhibit neuroprotective effects via Nrf2-mediated antioxidant, anti-inflammatory, and mitochondrial functional/biogenetic effects.	[48]
	Mice treated with a viral vector expressing human α-SYN	100 and 300 mg/kg DMF (i.g.)	DMF prevented Synucleinopathy in a mouse model of PD by activating Nrf2 signaling	[49]
	MPTP-treated mice	10, 30, and 100 mg/kg DMF (i.g.)	DMF protected against experimental PD via regulation of the NF-κB/Nrf-2 pathway	[50]

Disease condition	Experimental model	Effective dose	Outcomes	References
Retinal degeneration	I/R injury in mice	50 mg/kg MMF (i.p.)	MMF reduced retinal I/R injury in mice via induction of Nrf2 signaling	[51]
	Light-induced retinal damage in mice	100 mg/kg MMF (i.p.)	MMF-mediated HCAR2 signaling provided neuroprotection via reduced microglial activation, inflammation, and oxidative stress.	[52]
Sepsis	Rats subjected to cecal ligation and puncture procedure	15 mg/kg of DMF (i.g.)	DMF reduced inflammation and oxidative stress in heart, liver, lung, kidney, and brain, and improved cognitive function	[53, 54]
		50 mg/kg MMF (i.p.)	MMF alleviated sepsis-induced hepatic dysfunction by reducing oxidative and inflammatory via the inhibition of the TLR-4/ NF-κB signaling pathway.	[55]
Sickle cell disease	HbSS-Townes and NY1DD mice	30 mg/kg DMF (i.g.)	DMF increased expression of nuclear Nrf2 in the liver and kidney to decreases oxidative stress and inflammation	[56]
Sickle cell retinopathy	HbSS-Townes mice	1 mM (intravitreal) and 15 mg/ ml MMF (in drinking water)	MMF treatment-induced fetal hemoglobin production and reduced oxidative stress and inflammation via Nrf2 activation	[34, 35]
Spinal cord injury	SCI injury in mice using aneurysm clip	30 mg/kg (i.g.)	DMF and MMF improved SCI injury in mice.	[57]
Ulcer	Rats exposed to chronic foot-shock stress	2.5 and 5 mg/ kg MMF (i.p.)	MMF restored monoamine, corticosterone, and cytokine homeostasis by regulating neuroendocrine-immune systems	[39]

Table 1.
Some important in vivo *studies showing the use of fumaric acid esters for the treatment of oxidative stress and inflammation.*

4.1 Gastrointestinal diseases

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine that includes Crohn's disease and ulcerative colitis [58, 59]. Treatments for IBD range from symptomatic treatment with anti-diarrheal medications, anti-inflammatory agents or immunosuppressive drugs to more radical surgical interventive strategies (e.g. partial or complete colectomy). These strategies are effective in a number of patients however given the complex etiology of IBD, the need for new and/or improved therapeutic strategies remains high. Given the well-established link between inflammation and IBD development and progression, it is not surprising that several groups have sought to test the efficacy of FAE in this condition. For the most part, these studies have been conducted using experimental models of colitis; rodents treated with dinitrobenzene sulfuric acid (DNBS) or dextran sodium sulfate (DSS), etc. [60, 61]. Casili et al. induced colitis in mice via intrarectal administration of DNBS (4 mg/mouse). DMF (10, 30 or 100 mg/kg) was then administered orally

every 24 h, starting 3 h after the administration of DNBS and continuing over the course of 4 days. DMF treatment to DNBS treated mice significantly improved colon injury and histological score. Further DMF also reduced lipid peroxidation by regulating the expression of SOD2 (superoxide dismutase 2, mitochondrial) and Nrf2. The anti-inflammatory effect of DMF was evident by a reduction in the expression of TNF-α (tumor necrosis factor- α), IL-1β (Interleukin 1 beta) and ICAM-1 (intercellular adhesion molecule 1) and P- selectin. This effect was thought to be a result of reduced IκB-α degradation to prevent nuclear translocation of p65 NF-κB (Nuclear factor-κB). Moreover, *in vitro* DMF treatment improved hydrogen peroxide-induced barrier dysfunction of human intestinal epithelial cells. The authors also confirmed the protective effect of DMF on experimental colitis using another model (9-week-old IL-10KO mice). Collectively, this study demonstrated that DMF could reduce experimental colitis by regulating inflammation and oxidative stress [38]. In another study, Liu et al., 2016 evaluated the efficacy of DMF in reducing DSS-induced murine colitis. Wild-type and $Nrf2^{-/-}$ mice received either vehicle or 3% (w/v) DSS in drinking water for 7 days and thereafter provided with only drinking water for another 3 days. Groups of mice were also given 30 or 60 mg/kg DMF (i.g.) from day 1 to 10. DMF treatment significantly reduced oxidative stress and inflammation and thereby improved signs/symptoms of colitis in DSS-treated mice. However, these effects were lost in $Nrf2^{-/-}$ mice, highlighting the importance of the Nrf2-mediated mechanism of action of the drug. This was supported by additional *in vitro* studies in which the authors showed that DMF-mediated Nrf2 activation reduces NLRP3 (NLR family pyrin domain containing 3) inflammasome activation to control intestinal inflammation.

Consistent with the above gastrointestinal benefits derived from DMF/MMF treatment, the efficacy of MMF treatment in improving stomach ulcers in rats has also been described. Although the detailed mechanism of action was not evaluated, the authors attributed the protective effect of the compound in this condition to be due primarily to the anti-inflammatory activity of MMF [39]. Collectively, these studies indicate that DMF/MMF therapy may be of benefit the clinical management of inflammatory gastrointestinal disorders. This is interesting given that gastrointestinal (GI) side effects (e.g., nausea, vomiting, diarrhea, and upper abdominal pain) are one of the most commonly reported complaints in patients receiving DMF therapy [62, 63]. Indeed, during phase 3 clinical trials for multiple sclerosis, adverse events (AEs) involving the GI system were reported in 40% of patients treated with DMF compared with 30% of patients treated with placebo [64, 65]. Though the adverse GI events are generally mild in severity and typically resolve within the first 2 months of treatment, these issues may impact patient quality of life and ultimately medication adherence. Thus, while a number of experimental studies have reported gastroprotective effects of DMF, there is some concern as to whether such therapy could reliably be extrapolated to clinical management of gastrointestinal disorders in human patients. However, the increasing number of additional reports of DMF/MMF benefit in the digestive system that continue to arise in the scientific literature suggests that perhaps efforts to implement DMF/MMF therapy for use in this regard should not be dismissed completely. For example, Rao and Mishra [66] performed a preliminary study demonstrating the hepatoprotective effects of MMF isolated from *Fumaria Indica* extract in various models of hepatotoxicity. Although the study was preliminary and had some limitations, it does introduce a possible hepatoprotective effect of MMF. This is supported also by a recent study by Abdelrahman et al. [67] that reported the protective effects of DMF treatment on acetaminophen-induced hepatic injury in mice. Acetaminophen-treated mice receiving a single or double dose of DMF (100 mg/kg) showed reduced oxidative stress, inflammation, and associated liver damage compared to non-DMF treated

animals. Hence, additional studies in larger animal models and at some point, in humans, to test, develop and/or refine DMF/MMF formulations to improve potential suitability for use in the treatment of gastrointestinal or liver diseases are warranted.

4.2 HIV-induced neuroinflammation and neurotoxicity

With improvements in treatments for HIV (human immunodeficiency virus), lifespan has increased significantly affected persons. However, neuroinflammation and/or toxicity remain major concerns in this disease. The critical relevance of neuroinflammation to the etiology of MS, a disease for which DMF/MMF therapy is already approved, is undeniable [68]. Further, patients with MS are at considerably higher risk for neurotoxicity than are patients without the demyelinating disease [69]. Given these commonalities between MS and HIV-induced neurologic disease, preclinical testing of DMF/MMF in the latter is of interest. Using an *in vitro* model of HIV-mediated neurotoxicity, Cross et al. 2011 [70] showed that HIV infection dysregulates macrophage antioxidant response and reduces the expression of heme oxygenase-1 (HO-1). Importantly, DMF and MMF (5–30 μM) dose-dependently suppressed HIV replication, improved antioxidant response and reduced neurotoxin release, effects that the authors proposed to be mediated via a two-way action of DMF: (1) inhibition of NF-kB nuclear translocation and consequent suppression of HIV replication, and (2) decreased neurotoxin release stemming from HO-1 induction. Further, they also found that DMF reduces CCL2 (C-C Motif Chemokine Ligand 2)-induced monocyte chemotaxis, suggesting that DMF additionally decreased the recruitment of activated monocytes to the CNS (central nervous system) in response to inflammatory mediators. Based on the above, the authors concluded that dysregulation of the antioxidant response during HIV infection drives macrophage-mediated neurotoxicity and DMF could serve as an adjunctive neuroprotectant. In a separate study, Ambrosius et al. [71] evaluated the effect of MMF on microglia activation and subsequent neurotoxicity. MMF treatment (10–30 μM) significantly reduced HIV-mediated neurotoxicity in microglia cells (**Figure 3**). A similar but prior study by a different group showed MMF to be capable of inducing a phenotypic shift from pro-inflammatory to anti-inflammatory macrophages [72] however, Ambrosius et al. did not observe such effects. These differences could be model-dependent or related to methodological differences in the two studies and therefore require further investigation since the authors did not comprehensively evaluate the possible mechanism of action in these short reports. Notwithstanding, however, the opposing effects of DMF/MMF on microglial responses, particularly those of an inflammatory nature, appear to be solidly supported by several other studies [30] which in turn, collectively support additional effort to advance DMF/MMF therapy for potential use in HIV-associated neuroinflammation and toxicity.

4.3 Nephrotoxicity

Very little information exists on the protective effect of FAEs on renal function. A study by Takasu et al. [42] evaluated the effect of DMF treatment on CsA (calcineurin inhibitor)-induced nephrotoxicity. Male *Sprague–Dawley* rats were treated with 20 mg/kg CsA or CsA + 50 mg/kg DMF (i.g.) for 28 days. At the end of the treatment schedule, renal function, histopathology, malondialdehyde (MDA), myeloperoxidase levels, and antioxidant enzyme expression were determined. DMF co-treatment ameliorated CsA-induced renal dysfunction as evidenced by a significant decrease in serum creatinine and urea levels, as well as improvement of creatinine clearance. DMF also significantly decreased serum and renal MDA

and myeloperoxidase contents whereas, protein expression of NQO-1 (NAD (P) H quinone oxidoreductase-1), a major cellular antioxidant and the detoxifying enzyme, was significantly enhanced by DMF administration. Although evidence is limited, the above study supports the protective potential of DMF/MMF therapy in a clinically relevant model of nephrotoxicity, an effect that is afforded in part via DMF's robust ability to enhance the cellular antioxidant capacity and thereby, inhibit oxidative stress and inflammation [42] as described in other cell and tissue systems. Thus, while much remains to be learned about the possible use of DMF/MMF in the treatment of renal diseases, initial results are encouraging.

4.4 Non-HIV related neurotoxicity

Prior discussion (subSection 4.2) of neurotoxicity in this chapter was related specifically to that occurring in HIV. Irrespective, however, of the mitigating disease or pathologic process, the brain is indisputably sensitive to pro-inflammatory and/or oxidative insult. Hence, neurotoxicity can emanate from multiple variable causes. Kume et al. [43] evaluated the ability of DMF to protect against *in vitro* and *in vivo* oxidative stress in the central nervous system induced via pro-oxidant agents like sodium nitroprusside and hydrogen peroxide (H_2O_2). DMF pretreatment (60–200 mg/kg) for 24 h dose-dependently protected against 10 nM sodium nitroprusside-induced brain damage and in rat primary striatal cell cultures, 10 μM DMF markedly prevented cytotoxicity stemming from exposure of cells to H_2O_2 (1 mM). Interestingly, the protective effects of DMF against *in vitro* oxidative stress were countered by the HO-1 inhibitor zinc protoporphyrin IX however, buthionine sulfoximine, an inhibitor of glutathione synthesis, did not interfere with the protection afforded by DMF. Collectively, these results support the potential of DMF/MMF therapy in conditions of neurotoxicity and suggest that its ability to activate HO-1 may be critical. Neural stem/progenitor cells (NPCs) are a heterogeneous population of self-renewing and multi-potent cells that can differentiate into neurons, astrocytes, or oligodendrocytes (post-mitotic daughter cells) [73, 74]. Hence, the survival of these cells could greatly impact various forms of neurodegenerative diseases. Wang et al. [75] reported on the neuroprotective effects of DMF on mouse and rat neural stem/progenitor cells (NPCs) and neurons. DMF treatment reduced reactive oxygen species (ROS) production, increased the frequency of the multipotent neurospheres and enhanced the survival of NPCs following H_2O_2-mediated oxidative stress. DMF also decreased oxidative stress-induced apoptosis and promoted the survival of motor neurons, effects that this group demonstrated to be mediated via the Nrf2-ERK1/2 MAPK pathway. These studies provide additional support of the overwhelmingly protective effects of FAE in multiple brain cell types and therefore, of the potential feasibility of this therapy in the prevention and treatment of neurodegenerative diseases.

4.5 Pancreatitis

Chronic pancreatitis (CP) is a progressive inflammatory disorder that results in the destruction and fibrosis of the pancreatic parenchyma and its endocrine and exocrine dysfunctions [76]. Various research groups have evaluated the effect of DMF treatment on acute and chronic pancreatitis. In one of the studies, chronic pancreatitis in rats was induced by five injections of 250 mg/100 g L-Arginine and sacrificed 7 weeks later. In another group 25 mg/kg DMF was given orally 24 before L-arginine treatment and continued thereafter until the end of the study. DMF treatment significantly improved glucose tolerance, pancreas histology, biochemical parameters (MDA and MPO; myeloperoxidase), and induced HO-1 expression [44].

However, this study did not evaluate the mechanism of action for DMF-induced protection. Another study by Robles et al. [45] evaluated the efficacy of DMF in an acute model of pancreatitis. Acute pancreatitis was induced by two injections of 3 g/kg L-Arginine (1 hr. apart) to rats and sacrificed later at 24 and 72 hr. DMF (25 mg/kg) was orally administered to rats 24 h before L-arginine and continued until sacrifice. The histology of the pancreas was significantly improved in DMF-treated animals possibly due to decreased cleaved caspase-3 (apoptosis) and MDA levels. This group additionally stimulated splenocytes with 1 μg/ml for 24 h with or without DMF 20 μM. *In vitro* DMF treatment significantly reduced proinflammatory cytokine secretion in rat splenocytes, although a definitive mechanism for this DMF-mediated action was not put forward. Recently, however, Zhang and colleagues [46] too evaluated the effect of DMF on L-arginine induced chronic pancreatitis. In brief, this group treated *Wistar* rats intraperitoneally with L-arginine 5 times (250 mg/100 kg, twice per time, each interval of 1 h) to induce chronic pancreatitis (CP). One group of rats was treated with 20 mg/kg DMF. Compared with control (untreated) group, the weight of rats in CP group was significantly reduced at weeks 2, 4 and 6; blood glucose levels were significantly increased, the histopathological scores of pancreatic atrophy, acinar injury, edema, and cellular infiltration increased, levels of MDA and MPO increased, and the islet equivalent and islet activity decreased at 0, 30, 60, 120 and 180 min., parameters that were all prevented or reversed in the DMF-treated CP group. Thus, DMF treatment can protect against CP induced by L-arginine and islet function in rats. Although these three studies support the potential of DMF/MMF therapy in pancreatitis, the exact mechanism (s) to explain the benefits attained remains unknown. Because therapies to impact pancreatitis are extremely limited at present, additional detailed studies to test the efficacy of FAE in this condition would certainly be worthwhile in hopes that findings emanating therefrom could be carried forward to use in a clinical setting.

4.6 Parkinson's disease

Again, the brain is especially sensitive to perturbations caused by oxidative and/or inflammatory stress. In fact, these factors, particularly oxidative stress, are central to the pathology of several neurodegenerative diseases, including Parkinson's disease (PD) [77, 78] therefore, therapies designed to enhance antioxidant potential and counter this stress may be of clinical value [79, 80]. Scientific studies published within the last couple of years highlight the high clinical potential the repurposing of DMF/MMF for the treatment of PD holds. Using various *in vitro* and *in vivo* studies it has been demonstrated that DMF/MMF induced Nrf2 signaling can protect against oxidative stress and inflammatory conditions related to PD. In an initial study by Jing et al. [47], DMF (2–4 μM) pre-treatment significantly reduced hydroxydopamine (6-OHDA) induced generation of ROS and subsequent cytotoxicity in SH-SY5Y cells. The increase in ROS production caused by 6-OHDA treatment was also attenuated by DMF. Further, siNrf2 treatment blocked DMF's protection against 6-OHDA-induced neurotoxicity. *In vivo*, oral administration of DMF (50 mg/kg) to C57BL/6 mice up-regulated expression of Nrf2 and Nrf2-dependent cytoprotective genes. Taken together, this study provided initial evidence for the protective role of DMF in PD. This was followed by three different studies focusing on the mechanism of action for DMF and its metabolite, MMF in mediated protection against PD. Ahuja et al. [48] compared the effects of DMF and MMF on Nrf2 signaling by evaluating its ability to block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced experimental PD. Their results showed that Nrf2 activation by DMF was associated with depletion of glutathione, decreased cell viability, and inhibition of mitochondrial oxygen consumption and glycolysis

rates in a dose-dependent manner. Contrary to this, MMF increased these activities *in vitro*. However, both DMF and MMF activated the Nrf2 pathway via S-alkylation of the Nrf2 inhibitor Keap1 which promoted the nuclear exit of the Nrf2 repressor Bach1 to improve mitochondrial biogenesis. Despite the *in vitro* differences, both DMF and MMF exerted similar neuroprotective effects and blocked MPTP neurotoxicity in wild type but not in *Nrf2*$^{-/-}$ mice. It was concluded that DMF and MMF exhibit neuroprotective effects because of their distinct Nrf2-mediated antioxidant, anti-inflammatory, and mitochondrial functional/biogenetic effects, but MMF does so without depleting glutathione and inhibiting mitochondrial and glycolytic functions. Therefore, the authors advocated for the possible development of MMF rather than DMF as a novel therapy for PD. Synucleinopathies (also called α-synucleinopathies; α-SYN) are neurodegenerative diseases characterized by the abnormal accumulation of aggregates of alpha-synuclein protein in neurons, nerve fibers or glial cells [81]. Lastres-Becker et al. [49] conducted a study in which they focused primarily on the role of DMF in regulating synucleinopathies associated with oxidative stress and inflammation. In brief, an adeno-associated pseudotype 6 (rAAV6) viral vector was used to express human α-SYN under the neuron-specific human synapsin 1 promoter to create conditions of PD and animals were treated daily with DMF (100–300 mg/kg) via oral gavage. DMF protected nigral dopaminergic neurons against α-SYN toxicity and decreased astrocytosis and microgliosis. However, this protective effect was not observed in *Nrf2*$^{-/-}$ mice. Additionally, *in vitro* studies indicated that the neuroprotective effect was correlated with altered regulation of autophagy markers and with a shift in microglial dynamics toward a less pro-inflammatory and a more wound-healing phenotype (**Figure 3**). These experiments provide a compelling rationale for targeting Nrf2 with DMF as a therapeutic strategy to reinforce endogenous brain defense mechanisms against PD-associated synucleinopathy. These findings are supported by another study in which daily oral administration of DMF (10, 30, and 100 mg/kg) significantly reduced neuronal cell degeneration of the dopaminergic tract and behavioral impairments induced by four injections of the dopaminergic neurotoxin MPTP. Moreover, treatment with DMF prevented dopamine depletion, increased tyrosine hydroxylase, and dopamine transporter activities, and also reduced the number of α-synuclein-positive neurons. Furthermore, DMF treatment up-regulated Nrf2 as evidenced by the increased activation of SOD2 and HO-1 and elevated levels of glutathione, and increased $NeuN^{+}/Nrf2^{+}$ cell number in the striatum. Moreover, DMF reduced IL-1β levels, cyclooxygenase 2 activities, and neuronal nitrite oxide synthase expression. This treatment also modulated microglial activation (**Figure 3**), restored nerve growth factor levels, and preserved microtubule-associated protein 2 alterations. Using the Nrf2 inhibitor trigonelline, the authors were able to confirm the Nrf2 dependency of the protective mechanism. Collectively, these results demonstrated that DMF protects against experimental PD via NF-κB/Nrf2 pathway [50]. Several other antioxidants have shown potential as therapeutic options for PD, however, because DMF/MMF is already FDA-approved, the potential viability of this candidate therapy for PD is enhanced.

4.7 Retinal degenerative diseases

In recent years, others and we have investigated the potential of MMF in the treatment of degenerative retinal diseases. In an early study, we showed MMF to be protective against reactive gliosis, a characteristic response of Muller glial cells to an environment rich in pro-oxidant and inflammatory factors in retinal disease. Folate uptake by Muller cells is considered a key event in this process [82]. MMF treatment significantly reduced folate uptake by Muller cells by decreasing the expression and

activity of proton-coupled folate transporter (PCFT), a transporter integral to the uptake of folate. This was the first report demonstrating that MMF could regulate folate transport in retinal glial cells and therefore, be potentially useful in the treatment of degenerative retinal diseases. To determine whether, in addition to down-regulating pro-inflammatory mechanisms, MMF affects counteractive or protective signaling, in a subsequent study we evaluated also the effect of the compound on the expression and activity of the cysteine/glutamate exchanger SLC7A11 (system x_c^-), a transport system critical for the intracellular entry of the amino acid cysteine which is required for glutathione synthesis [28]. Glutathione is the most abundant endogenous antioxidant in the retina and is therefore essential for the protection of retinal cells against oxidative stress. Further, retinal pigment epithelial (RPE) cells are one of the highest producers of glutathione of any cell type in the body. As such, we exposed human retinal pigment epithelial (ARPE-19) cells to MMF in the presence or absence of pro-oxidant stimuli and evaluated the dose- and time-dependent effects on system x_c^- mRNA, protein, and activity levels. MMF was found to up-regulate each of these parameters and additionally, up-regulate hypoxia-inducible factor 1-alpha (Hif-1α), nuclear factor erythroid 2-related factor 2 (Nrf2) expression and increase total reduced glutathione (GSH) content. Collectively, our early *in vitro* studies demonstrated that MMF affects multiple pathways in multiple retinal cell types in a manner that is overall protective against oxidative damage.

We sought next to determine whether our findings extrapolate to the *in vivo* condition, therefore, we evaluated the efficacy of MMF in a living animal model of retinal disease. Retinopathy is a major cause of vision loss in sickle cell disease (SCD) and therapies to prevent and treat sickle retinopathy (SR) are very limited. Therapeutic induction of γ-globin expression and subsequent induction of fetal hemoglobin (HbF) production can alleviate some SCD-associated complications. Interestingly, Nrf2 inducers have been demonstrated to be effective γ-globin inducers [83]. The robust inductive properties of MMF on Nrf2 translocation and activity have been long recognized therefore, it was logical to explore the effects of MMF in SCD. Not only did we confirm that RPE cells, cells integral to retinal health and function, produce HbF but that MMF treatment of Townes humanized SCD mice of SCD resulted in reductions in the expression of pro-oxidant and inflammatory factors and turn, preserved retinal morphology [35]. Shortly after this study, Cho et al. [51] too reported on the potential benefit of MMF in the treatment of retinal disease in a mouse model of retinal ischemia-reperfusion. Specifically, they showed that MMF promotes Nrf2-neuroprotection in this model. MMF treatment was associated with significant increases in the expression of Nrf2-responsive antioxidant genes and a suppression of inflammatory responses as evidenced by increased expression of NAD (P) H quinone dehydrogenase 1, thioredoxin reductase 1 and heme oxygenase-1 along with decrease in interleukin-1β, chemokine (C-C motif) ligands (2, 7 and 12), expressions. Collectively, these molecular improvements interpreted to improved retinal function as evidenced by electroretinogram recordings performed on live mice and were heavily dependent upon the expression and activity of Nrf2.

Because these initial reports of MMF's potential efficacy in protecting against retinal degeneration were conducted acutely, we decided to evaluate the effect of long-term administration of the compound (5 months administration of 15 mg/ml MMF in drinking water) in the humanized SCD model [34]. Importantly we found via high-pressure liquid chromatography (HPLC) and hematological analyses of peripheral blood that MMF treatment reduced sickle hemoglobin (HbS) content and white blood cell counts, and improved hematocrit, red blood cell number, and hemoglobin concentrations significantly in SCD mice. In retina specifically, the mRNA and protein expression of well-established markers of inflammation and oxidative stress (i.e., vascular endothelial growth factor, intercellular adhesion molecule-1,

interleukin-1β, dihydroethidium labeling) was reduced and the development and progression of SCD-like retinal pathology in these mice were ameliorated. Additional related *in vitro* studies performed toward elucidating the molecular mechanisms responsible for the MMF-induced improvements that were observed implicate Nrf2 and Bcl11A (B-cell lymphoma/leukemia 11A) as key players. This study was of extreme significance because not only did it support strongly the notion that fumaric acid ester therapy may be of benefit for the treatment of retinal pathology, especially in SCD, but for SCD in general, a concept that we have since patented [84]. Perhaps equally as astounding is the fact that MMF delivered systemically induced such robust effects in retina, meaning that MMF must be capable of crossing in significant quantities or otherwise inducing signaling across the blood-retinal barriers. Given the known difficulties with non-invasive yet efficacious drug delivery to the posterior segment of the eye (retina) and the commonality of oxidative stress and inflammation as key causative factors in the development and progression of numerous retinal diseases, the clinical relevance and therefore potential impact of the above findings is extremely high. Indeed, new reports of potential benefit derived from MMF in animal models of the degenerative retinal disease continue to surface, such as the recent study by Jiang et al. [52] demonstrating that MMF treatment protects against light-induced retinal damage on BALB/C mice and effect due potentially to HCAR2-dependent signaling in retinal microglia cells (**Figure 3**). Eventually, data emanating from these preclinical reports may spur increased interest in moving toward clinical testing and implementation of FAE therapy in the near future.

4.8 Sepsis

Sepsis is a potentially fatal illness that can lead to the damage of multiple organs [85]. The condition is deeply associated with oxidative stress and inflammation. Firstly, a study by Giustina et al. [53] reported the protective effects of DMF against multi-organ sepsis by modulating oxidative stress and inflammation. It was reported that oral administration of 15 mg/kg of DMF provides significant protection against sepsis-induced multi-organ (heart, liver, and lung) damage in rats. Later, the same research group reported the protective effects of DMF treatment on sepsis-associated inflammation and oxidative stress and cognitive impairment in the brain [54]. Although both these studies were descriptive in nature as neither evaluated in detail the underlying mode of action, they provide evidence that DMF might be used successfully for the clinical management of sepsis. This is supported by a study by Shalmani et al. [55] in which it was reported that 50 mg/kg (i.p.) MMF treatment improved sepsis-induced liver dysfunction by regulating the TLR-4/NF-κB signaling pathway. Collectively, these preclinical studies provide a great foundation for future clinical evaluations of the utility of FAE in the management of organ damage in sepsis.

4.9 Sickle cell disease-associated oxidative stress and inflammation

Uncontrolled hemolysis and subsequent release of hemoglobin (Hb) and heme into the vasculature is a hallmark of sickle cell disease (SCD) [86, 87]. Heme, a damage-associated molecular pattern, is highly pro-oxidative and proinflammatory and induces vaso-occlusion in murine models of sickle cell disease (SCD) [88]. A study by Belcher et al. evaluated the protective effect of DMF treatment on SCD associated oxidative stress and inflammation in the liver and kidneys [56]. DMF (30 mg/kg/day) or vehicle (0.08% methylcellulose) was administered for 3–7 days to NY1DD and HbSS-Townes SCD mice. DMF had a significant reductive impact on vaso-occlusion in SCD mice. It increased the nuclear translocation

of Nrf2 and cellular mRNA of Nrf2-responsive genes in livers and kidneys, and increased heme defenses, including HO-1, haptoglobin, hemopexin, and ferritin heavy chain, without altering plasma Hb and heme levels. Markers of inflammation were also reduced. Interestingly, much of the DMF-induced benefit was blunted by the HO-1 inhibitor, protoporphyrin. Chronic treatment (24 weeks) of SCD with DMF decreased hepatic necrosis, inflammatory cytokines, and irregularly shaped erythrocytes, and increased HbF but did not alter hematocrit, reticulocyte counts, lactate dehydrogenase or plasma heme levels or, spleen weights. These results [56] together with our previously highlighted findings in SCD (subSection 4.7) [34, 35], are supportive of the multiple beneficial effects of DMF/MMF on the pathogenesis of SCD and the need for further clinical evaluation of the drug for this indication.

4.10 Spinal cord injury

Patients with spinal cord injury (SCI) usually have permanent and often devastating neurologic deficits and disabilities. The currently available therapeutic options include surgical decompression, methylprednisolone and hemodynamic control [89, 90]. Hence, the development of a new therapy for SCI holds great merits. Recent work by Cordaro et al. [57] evaluated the beneficial effects of DMF and MMF in a mouse model of traumatic SCI. Using an aneurysm clip, SCI was induced by extradural compression of the spinal cord at T6-T7 for 1 min. Mice were then treated with 30 mg/kg (i.g) DMF or MMF one and 6 h post-SCI. To evaluate the locomotor activity, study mice were treated with DMF/MMF once daily for 10 days. It was observed that mice treated with DMF exhibited a significant and sustained recovery of motor function. DMF/MMF significantly reduced the severity of inflammation by modulation of pro-inflammatory cytokines and apoptosis factors and increased neurotrophic factors. The authors concluded that the observed results were attributable to reduced secondary inflammation and tissue injury and therefore, DMF may constitute a promising target for future SCI therapies [57]. This study provided the first scientific evidence for the protective role of DMF in the treatment of SCI, however, additional detailed experimental and preclinical studies are needed to identify the potential mechanism(s) of action and enhance the likelihood that this therapy could be advanced to clinical testing and implementation.

4.11 Stroke

Over the past 2 years, researchers worldwide have published several articles on the role of FAE in the treatment of stroke. In one of the early studies on intracerebral hemorrhage (ICH), male rats and mice (including *Nrf2*-deficient animals) were subjected to intracerebral injection of blood and then treated with DMF [40]. In rats, 5 mg/kg DMF was administered at 2 h post-ICH and again orally twice a day on days 1–3, whereas in mice, the same dose of DMF was injected (i.p.) 24 h post-ICH and then at days 2 and 3. Treatment with DMF induced Nrf2-target genes, improved hematoma resolution, reduced brain edema and eventually enhanced neurological recovery in rats and wild type mice, but not in *Nrf2*$^{-/-}$ mice. Based on these findings, the authors proposed that DMF may offer an impressive 24 h therapeutic window of opportunity in which to treat ICH, a concept certainly worthy of further evaluation. The potential of DMF/MMF therapy in ICH is supported further by work by Iniaghe et al. [41] in which male CD-1 mice were subjected to intrastriatal infusion of bacterial collagenase, autologous blood or sham surgery. After ICH, animals either received vehicle, DMF (10 mg or 100 mg/kg) or casein kinase 2 inhibitor (E)-3-(2,3,4,5-tetrabromophenyl) acrylic acid (TBCA). Some mice also received scrambled siRNA or MAFG siRNA 24 h before ICH. DMF

treatment reduced Evans blue dye extravasation, decreased brain water content, microglia activation (**Figure 3**), ICAM-1 expression and, improved neurological deficits and casein kinase 2 levels. Interestingly, TBCA and MAFG siRNA blunted protection afforded by DMF. Hence, it was concluded that DMF reduced inflammation, blood-brain barrier permeability, and improved neurological outcomes via casein kinase 2 and Nrf2 signaling pathways in mice.

Similar to other neurodegenerative disorders, oxidative stress is common also to the pathogenesis of ischemic stroke, potentiating the neuronal malfunction and cell death characteristic of this disease [91]. Given that the up-regulation of antioxidant genes through activation of the Nrf2 is one of the key mechanisms of cellular defense against oxidative stress [92], it is logical to explore the efficacy of FAE therapy in this condition. Congruent with this, three additional groups used experimental models of ischemic stroke to evaluate the efficacy of FAEs. In 2016, Lin et al. [36] observed that MMF (25–100 μM) rescued cultured cortical neurons from oxygen–glucose deprivation (OGD) and suppressed pro-inflammatory cytokines produced by primary mixed neuron/glia cultures subjected to OGD. In rats, DMF treatment (25 or 50 mg/kg twice daily) significantly decreased infarction volume by nearly 40% and significantly improved neurobehavioral deficits after middle cerebral artery occlusion (MCAO). In the acute early phase (72 h after MCAO), DMF induced Nrf2 expression and its downstream mediator HO-1. In addition to its antioxidant role, DMF also acted as a potent immunomodulator, reducing the infiltration of neutrophils and T-cells as well as the number of activated microglia/ macrophages in the infarct region. Concomitantly, levels of pro-inflammatory cytokines were greatly reduced in the plasma and brain and oxygen–glucose deprived neuron/glia cultures. Further, using a mouse model of transient focal brain ischemia, Yao et al. [37] showed that DMF and MMF (30 mg/kg i.p.) significantly reduced neurological deficits, infarct volume, brain edema, and cell death. Additionally, DMF and MMF suppress glial activation following brain ischemia. Importantly, the protection of DMF and MMF was most evident during the sub-acute stage and was abolished in *Nrf2*$^{-/-}$ mice, indicating that the Nrf2 pathway is required for the beneficial effects of DMF and MMF [37]. In another study, murine organotypic hippocampal slice cultures, and two neuronal cell lines were treated with DMF and MMF [93]. The ischemic condition was generated by exposing cells and slice cultures to oxygen-glucose deprivation. Treatment with both DMF and MMF (30–100 μM) immediately upon reoxygenation strongly reduced cell death in hippocampal cultures *ex vivo*. Both DMF and MMF promoted neuronal survival in HT-22 and SH-SY5Y cell lines exposed to ischemic stress. However, interestingly, DMF but not MMF activated the anti-oxidative Nrf2 pathway in neurons. Accordingly, the protective effect of DMF but not MMF was abrogated in the neurons of Nrf2-deficient mice. These results provide the basis for a new therapeutic approach to treat ischemic pathologies such as stroke using a drug that is already approved by US-FDA for clinical use.

5. Safety profile

By and large, the short-term safety profile for DMF in patients with RMS is highly favorable [64, 65] and long-term safety analyses from the ENDORSE study sustains a favorable benefit: risk ratio [94]. The most common adverse events observed in patients receiving DMF include flushing, gastrointestinal (GI) events (e.g., diarrhea, nausea, abdominal pain, and vomiting), proteinuria, and pruritus [64, 65]. Aspirin pretreatment has been shown to reduce DMF induced adverse GI events [95]. Additionally, the leukotriene-receptor antagonist montelukast has been shown to help as well [96]. Further, it has been observed that consuming a high fat

and high protein meal just before DMF administration may reduce GI and flushing side effects by delaying its intestinal absorption. Notably, the risk of lymphopenia is higher in adults older than 55 years, in those with lower baseline lymphocyte counts, and those switching from natalizumab [97]. Cases of multifocal leukoencephalopathy (PML) following DMF treatment have also been reported [98–107]. Highly worthy of mention, however, is the fact that each of the affected patients detailed above had well-known pre-existing risk factors for PML including lymphocytopenia, sarcoidosis, cancer history, and/or prior efalizumab use. Thus, the negative effects of MMF treatment on PML should be interpreted very carefully. Like other pharmacological therapies, DMF/MMF treatment is associated with some side effects importantly however, advancements toward developing improved formulations minimize these events without losing efficacy are already being realized. For example, Alkermes, Inc. has developed diroximel fumarate (DRF), also known as ALKS8700, a novel MMF prodrug. Importantly, this new formulation has been shown to yield bioequivalent levels of MMF at the cellular level when compared directly to DMF (**Figure 1**) [108] while interacting less with off-target proteins and therefore producing fewer unwanted side effects [109]. Indeed, interim findings from EVOLVE-MS-1 and EVOLVE-MS-2 which demonstrate that DRF has a favorable safety and efficacy profile and is well-tolerated in MS patients [108, 110].

6. Conclusions

Drug repurposing is a very viable therapeutic strategy [18]. Many agents approved for other uses already have been tested in humans, so detailed information is available on their pharmacology, formulation and potential side effects. Since repurposing expands upon past innovative endeavors, hopeful new treatments could be prepared for clinical trials rapidly. Historically, pharmaceutical companies have achieved a number of successes via drug repositioning (e.g., for Viagra, thalidomide, metformin, etc.). Based on the literature available, DMF/MMF has been shown to protect against a variety of diseases other than MS and psoriasis.

7. Future perspectives

FAE are perhaps most noted for the robust antioxidant effects that they elicit via Nrf2 induction. A number of additional (non-FAE based) Nrf2 inducing drugs have been developed and tested in experimental and clinical systems in recent years (e.g., resveratrol, sulforaphane, etc.) and several have been with considerable success with regard to potential for clinical development [111]. However, the multimodal actions of FAE make this emerging drug stand out among the rest. It is commonly said that oxidative stress and inflammation go hand-in-hand, meaning that one potentiates the other in somewhat of a cyclic manner. Thus, it can only be hoped that in turn, if one is suppressed then the other similarly complies. However, things are usually not that simple. In the case of FAE, there are two arms of action: one induces Nrf2 and the other interacts with the anti-inflammatory hydroxycarboxylic acid receptor (HCAR2 or HCA2; **Figure 2**). Thus, the compound has a direct impact on inflammation independent of its actions on oxidative stress. The fascinating thing about these two mechanistic arms, is that they appear to act simultaneously in many cell and tissue systems. This may explain why FAE has exceled in so many variable pathologic conditions. MMF through its interaction with HCAR2, which is expressed by primary immune cells and a multitude of accessory immune cells (i.e., those that initiate the immune response and those cells like retinal pigment

epithelial cells, for example, that aren't truly "immune" cells but are capable just the same of secreting pro- and anti-inflammatory factors depending upon the stimulus), elicits a tremendous anti-inflammatory response. The combined Nrf2-inducing and immune-modulatory properties of FAE have enabled this drug to be efficacious in a broad range of body systems. The evidence provided in this chapter alone demonstrates convincingly that the benefits of FAE have been realized in the central nervous system (brain and retina), the cardiovascular system, the digestive and/or gastrointestinal system, the immune system, the integumentary system and the renal system; this list continues to grow. Thus, the potential clinical impact of FAE therapy use is high and importantly extremely broad. It is acknowledged that as with virtually all pharmacologic agents, FAE therapy is not without adverse effects. Importantly, however, the effects are relatively mild and the benefit(s) indisputably outweigh the risks. As such, there is a prompt need for additional experimental and clinical studies to translate the information gleaned from exploratory trials of FAE therapy in various cell, tissue, and disease types into clinical use.

Acknowledgements

We would like to acknowledge funding support from the National Eye Institute grants EY022704 and EY029113 to Pamela M. Martin.

Conflict of interest

P.M.M. is a coinventor of US20140171504 A1 patent titled "Methods of treating SCD and related disorders using fumaric acid esters." The remaining authors declare that they have no conflict of interest.

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