REVIEW ARTICLE

# Identifying the active pharmaceutical ingredient from a mixture of fumaric acid esters for the treatment of psoriasis: Hints from *in vitro* investigations

**Lilla Landeck[1*], Adriana Amasuno[2], Ignasi Pau-Charles[2], Khusru Asadullah[3]**

[1] Department of Dermatology, Ernst von Bergmann General Hospital, Potsdam, Germany
[2] Almirall S.A, Barcelona, Spain
[3] Department of Dermatology, University Hospital Charité Berlin, Germany

**Abstract:** A mixture of fumaric acid esters (FAEs) is approved for the oral therapy of psoriasis. However, for a long time the active ingredient of this mixture was unknown. We reviewed the *in vitro* data available for the different FAEs present in the multi compound drug and elaborate how they may contribute to possible clinical effects. Although helpful overall, many in vitro data must be viewed critically because the concentrations used in the experiments exceed the plasma levels reached in patients. The data suggest that dimethylfumarate (DMF) is the most active compound, mediating the major therapeutic effect after metabolization into monomethylfumarate (MMF) via an according receptor expressed on target cells. Identifying the active pharmaceutical ingredient within a mixture of compounds helps to subsequently eliminate unnecessary, potentially harmful compounds. This provides a promising example for an alternative precision medicine approach in clinical practice.

**Keywords:** dimethylfumarate (DMF); monoethylfumarate (MEF); monomethylfumarate (MMF); fumaric acid esters (FAEs); psoriasis

*Correspondence to: Lilla Landeck, Department of Dermatology, Ernst von Bergmann General Hospital Charlottenstrasse 72, 14467 Potsdam, Germany; E-mail: llandeck@klinikumevb.de

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## Introduction

Using personalized or precision medicine (PM) is commonly understood as an approach for the prevention and treatment of diseases that takes individual biological variability into account. Usually, this is achieved by applying biomarkers, enabling stratification of patients or individual dosing, for example. However, there is no formal uniform standardized definition for PM.

The European Union defines PM rather broadly as: to provide the right treatment to the right patient, at the right dose at the right time[1]. As a consequence, it might be important to identify the major active pharmaceutical ingredient within a mixture of compounds used in an approved drug. This allows unnecessary, or even potentially harmful, drug compounds to be eliminated.

In 1994, a proprietary combination of fumaric acid esters (FAEs) was licensed for the treatment of psoriasis by the German Drug Administration for use in Germany. Since then, fumarates have been established as one of the most commonly used oral treatments for moderate to severe psoriasis. The licensed FAE formulation contains dimethylfumarate (DMF), calcium, zinc, and magnesium salts of monoethylfumarate (MEF). While the clinical efficacy of this FAE mixture is well established, the combination of esters on which it is based, and its dosing regimen, were determined empirically. Since the mid-1990s, the modes of action and the contribution of the different FAEs to their overall therapeutic effect in psoriasis have been investigated in more detail. Here, we describe and compare the *in vitro* data for different FAEs that gave insight into the compound — DMF — that is

the major active ingredient accounting for the clinical effects in psoriasis.

## Pharmacological Activity of the FAEs in Psoriasis

Many *in vitro* and *in vivo* studies have attempted to clarify the mechanistic effects of each of the components of the approved FAE mixture (Fumaderm®), the drug most frequently used for oral therapy of psoriasis in Germany. Work to elucidate the roles of DMF and its main metabolite, monomethylfumarate (MMF), and MEF has been carried out in several different cell types and has provided a foundation of preclinical data on which to understand the effectiveness of DMF and the approved FAE mixture in the management of psoriasis[2–18]. Results of preclinical experiments with DMF, MMF and MEF are shown in Table 1.

However, the *in vitro* data have to be interpreted with caution, because the drug concentrations used in several experiments were high and often exceeded the concentrations reached in patients many-fold. Indeed, it seems that maximum concentrations ($C_{max}$) of fumarates in patients are usually within the range of 10–15µmol/L[19], whereas many preclinical studies have examined fumarate concentrations ≥40-fold this concentration (Table 1). In addition, the short *in vivo* half-life of fumarates needs to be considered. DMF is rapidly hydrolysed by esterases to MMF, the active metabolite, which is further metabolized into water and carbon dioxide[20]. DMF has a half-life of about 12 min[20], whereas that of MMF has been reported to be <40 minutes[19]. Peak concentrations of MMF are reported to occur between 2.5 and 6 hours[19,20].

## FAE Effects on Inflammatory Pathways

The effects of FAEs on inflammatory pathways have been studied in some depth. FAEs incubated with activated primary human peripheral blood mononuclear cells (PBMCs) have been reported to have differential effects on the secretion of inflammatory cytokines[13]. While DMF (1–100 µmol/L) and diethyl fumarate (DEF) exhibited potent suppression of tumour necrosis factor alpha (TNFα), interleukin (IL)-12 and type II interferon (IFNγ), fumaric acid (FA) and MEF (also known as ethylhydrogen fumarate [EHF]) did not display this inhibitory activity. Similarly, inhibition of IL-6, IFNγ and the keratinocyte growth factor transforming growth factor alpha (TGF-α), as well as stimulation of IL-10 secretion have been reported in activated human lymphocytes and keratinocytes co-cultured in the presence of DMF (but not MEF)[21]. In addition, inhibition of allo-reactive T-cell proliferation in a mixed leucocyte reaction was only observed in the presence of DMF and DEF[13]. The immunosuppressive effects of FAEs as demonstrated here were reported alongside a marked induction of heme oxygenase (HO-1), an anti-inflammatory stress protein. Induction of HO-1 and anti-inflammatory effects were blocked upon addition of glutathione (GSH), a known ligand of DMF. Furthermore, inhibition of HO-1 restored the previously diminished IL-12 and IFNγ production observed following treatment with FAEs[13]. More recently, further *in vitro* experiments in macrophages, PBMCs, HEK293 and HeLa cells have provided more evidence for the inhibitory effects of DMF (at concentrations ranging from 25–100 µM) on T-cytokine induction and its subsequent immunosuppressive activities (Table 1)[15,17,18].

Studies by Nibbering and colleagues in human granulocytes have reported several effects mediated by MMF, the main metabolite produced following DMF ingestion (Table 1). MMF actions included inhibition of formylated peptide-induced respiratory burst and enhanced cellular polarization, cAMP production and calcium mobilization[4,22]. MMF has also been shown to stimulate IL-4 and IL-5 in a dose-dependent manner when incubated with stimulated PBMCs; incubation with MMF had no effect on levels of IL-2, IFNγ or proliferative T-cell responses in these cultures[6]. Likewise, incubation of activated PBMCs and monocytes with MMF has been shown to stimulate activity of IL-10, TNF-α and IL-1 receptor antagonist (IL-1RA) independently of IL-12 secretion[23].

However, MMF activity has not been reported in all *in vitro* studies of this kind. In particular, MMF has shown no activity (compared with DMF) in studies that explored inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-dependent cytokine production by PBMCs[9,13], lymphocyte proliferation[13] or leucocyte-endothelial cell interactions[14].

Conversely, experiments in murine splenocyte cells have implicated DMF in the regulation of the NF-κB pathway and subsequent inflammatory pathways[9,24]. DMF has been shown to inhibit NF-κB driven production of cytokines and suppress translocation of p65 and p52 in a nuclear factor erythroid-derived 2 (Nrf-2)-independent manner. These effects were not seen with MMF or MEF. Modulation of the NF-κB pathway in this manner resulted in downstream suppression of inflammatory cytokine production, altered maturation and function of antigen-presenting cells, and immune deviation of T-helper cells (Th) from Th1 to Th17 profiles to a Th2 phenotype[9]. Changes in cytokine profile from a Th1 to Th2 phenotype, in combination with T-cell inhibition, have also been reported in humans[25,26].

*In vitro* studies in human endothelial cells have

**Table 1.** *In vitro* and *in vivo* effects of MEF, DMF and MMF on different target cells

| FAE compound | | Target cells | Cellular activities | Inhibition/stimulation | Reference |
|---|---|---|---|---|---|
| Reports of *in vitro* concentrations* | | | | | |
| MEF | 0.1–500 µg/mL | Cultured PHA-simulated human lymphocytes | Nucleic acid synthesis | Inhibition | Petres *et al*. 1975[3] |
| | | | Protein synthesis | Inhibition | |
| MMF | 200 mM/L | Granulocytes from healthy donors – *in vitro* | Respiratory burst | Inhibition | Nibbering *et al*. 1993[4] |
| DMF | 0.4–960 mM/L[a] | Human HaCaT cell line – *in vitro* | Proliferation | Inhibition | Sebök *et al*. 1994[5] |
| | | | DNA and protein synthesis | Inhibition | |
| MEF | 0.4–960 mM/L MEF-Zn | HaCaT cell line | Proliferation | No effect | |
| | 0.4–960 mM/L MEF-Ca | | | Inhibition[b] | |
| | 0.4–960 mM/L MEF-Mg | | | No effect | |
| MEF | 0.4–960 mM/L MEF-Zn | HaCaT cell line | DNA and protein synthesis | Inhibition | |
| | 0.4–960 mM/L MEF-Ca | | | Inhibition | |
| | 0.4–960 mM/L MEF-Mg | | | Inhibition | |
| MMF | 50–200 mM/L | Human lymphocytes from healthy donors | IL-4/IL-5 production | Stimulation | De Jong *et al*. 1996[6] |
| DMF | 100 mM/L | Human dermal microvascular endothelial cells | Expression of ICAM, VCAM and E-selectin | Inhibition | Vandermeeren *et al*. 1997 [7] |
| | 100 mM/L | Human umbilical vein endothelial cells (HUVEC) – *in vitro* | Expression of ICAM, VCAM and E-selectin | Inhibition | |
| MEF | 3–6 µg/mL | Human embryonic kidney 293 cells (HEK 293FT) – *in vitro* | Keap-1: modification of Cys residues | Limited effect (Cys151) | Brennan *et al*. 2015[8] |
| | 1–6 µg/mL | Human spinal cord astrocytes – *in vitro* | Nrf-2: nuclear translocation | Limited stimulation | |
| | 1–3 µg/mL | Human spinal cord astrocytes – *in vitro* | GSH levels | No effect | |
| DMF | 3–6 µg/mL | HEK 293FT cells – *in vitro* | Keap-1: modification of Cys residues | Stimulation (Cys 151, Cys257 and Cys273) | |
| | 1–6 µg/mL | Human spinal cord astrocytes – *in vitro* | Nrf-2: nuclear translocation | Stimulation | |
| | 1–3 µg/mL | Human spinal cord astrocytes – *in vitro* | GSH levels | Depletion | |
| DMF | 1–9 µg/mL | Primary human PBMCs and murine splenocytes from age-matched NRF2-/- mice or WT mice – *in vitro* | NF-κB-dependent cytokine production (e.g. IL-6, MIP-1β, IP-10) | Inhibition | Gillard *et al*. 2015[9] |
| MMF | 1–9 µg/mL | | | No effect | |
| MEF | 1–9 µg/mL | | | No effect | |
| Reports of *in vitro* dosages* | | | | | |
| MMF | 0.05–0.4 mM | Human juvenile keratinocytes (normal and SV-40-transformed) – *in vitro* | Intracellular free Ca release | Stimulation | Thio *et al*. 1994[10] |
| | 7–1000 µM | | Proliferation | Inhibition | |
| DMF | 0.05–0.4 mM | Human juvenile keratinocytes (normal and SV-40-transformed) – *in vitro* | Intracellular free Ca release | Stimulation | |
| | 7–1000 µM | | Proliferation | Inhibition | |
| MEF | 0.05–0.4 mM | Human juvenile keratinocytes (normal and SV-40-transformed) – *in vitro* | Intracellular free Ca release | Stimulation | |
| | 7–1000 µM | | Proliferation | Inhibition | |
| DMF | 5–30 µM/L | Co-cultured human keratinocytes and lymphocytes | IL-10 | Stimulation | Ockenfels *et al*. 1998[21] |
| | | | TGF-α | Inhibition | |
| | | | IFNγ | Inhibition | |

**Table 1.** *In vitro* and *in vivo* effects of MEF, DMF and MMF on different target cells (Continued)

| FAE compound | | Target cells | Cellular activities | Inhibition/stimulation | Reference |
|---|---|---|---|---|---|
| MMF | 100 µM | Cultured human PBMCs from psoriatic patients and healthy volunteers– *in vitro* | IL-10 | Stimulation | Asadullah *et al*. 1997[22] |
| | | | TNF-α | Stimulation | |
| | | | Il-1RA | Stimulation | |
| DMF | 1–319 µM | HaCaT cell line and normal human epidermal keratinocytes – *in vitro* | Expression of ICAM and HLA-DR | Inhibition | Sebök *et al*. 1998[11] |
| DMF | 3–100 µM | Human lympho-histiocytic cell line (U937) – *in vitro* | Apoptosis | Stimulation | Sebök *et al*. 2000[12] |
| MEF | 3–100 µM MEF-Zn | U937 lympho-histiocytic cell line | Apoptosis | Stimulation | |
| | 3–100 µM MEF-Ca | | | Stimulation | |
| | 3–100 µM MEF-Mg | | | No effect | |
| DMF | 1–100 µM | Cultured human lymphocytes – *in vitro* | Proliferation | Inhibition | Lehmann *et al*. 2007[13] |
| | 1–100 µM | Human PBMC from healthy donors – *in vitro* | Cytokine production (TNFα | Inhibition | |
| | 25–50 µM | Human PBMC from healthy donors – *in vitro* | HO-1 mRNA expression | Stimulation | |
| MMF | 1–100 µM | Cultured human lymphocytes – *in vitro* | Proliferation | No effect | |
| | 1–100 µM | Human PBMC from healthy donors – *in vitro* | Cytokine production (TNFα | No effect | |
| | 25–50 µM | Human PBMC from healthy donors – *in vitro* | HO-1 mRNA expression | No effect | |
| MEF | 1–100 µM | Cultured human lymphocytes – *in vitro* | Proliferation | No effect | |
| | 1–100 µM | Human PBMC from healthy donors – *in vitro* | Cytokine production (TNFα | No effect | |
| | 25–50 µM | Human PBMC from healthy donors – *in vitro* | HO-1 mRNA expression | No effect | |
| DMF | 5–200 µM | Human placental microvascular endothelial cells (HPEC-A2)/normal human lymphocytes – *in vitro* | Expression of ICAM, VCAM and E-selectin | Inhibition | Wallbrecht *et al*. 2011[14] |
| | | | Leukocyte/endothelial cell interactions (cell rolling and adhesion) | Inhibition | |
| MMF | 5–200 µM | Human placental microvascular endothelial cells (HPEC-A2)/normal human lymphocytes – *in vitro* | Expression of ICAM, VCAM and E-selectin | No effect | |
| | | | Leukocyte/endothelial cell interactions (cell rolling and adhesion) | No effect | |
| DMF | 25–100 µM | Bone marrow-derived murine macrophages – *in vitro* | Cytokine induction and gene transcription (Inc. TNF, IL-6, IL-10, GM-CSF) | Inhibition | McGuire 2016[15] |
| | 25–100 µM | HEK293 – *in vitro* | Downstream signalling | Inhibition | |
| | 50 µM | HeLa cells – *in vitro* | p38 MAPK activation | Stimulation | |
| MMF | 0–750 µM | Cultured primary murine keratinocytes | Nrf-2 expression | Stimulation | Helwa *et al*. 2017[16] |
| | | | HO-1 expression | Stimulation | |
| | | | AQP3 expression | Stimulation | |
| DMF | 10–20 mg/L | Human polymorphonuclear granulocytes from psoriasis patients – *in vitro* | NET expression | Inhibition | Hoffmann *et al*. 2017[29] |
| | | | ROS expression | Depletion | |
| | | | GSH levels | Depletion | |
| Reports investigating *in vitro* dosage and effect of *in vivo* dosing* | | | | | |
| MEF | 0.1–500 µg/mL | Cultured human lymphocytes | DNA synthesis | Inhibition | Hagedorn *et al*. 1975[2] |
| | | | Proliferation | Inhibition | |

**Table 1.** *In vitro* and *in vivo* effects of MEF, DMF and MMF on different target cells (Continued)

| FAE compound | | Target cells | Cellular activities | Inhibition/stimulation | Reference |
|---|---|---|---|---|---|
| DMF | 70 µM | PBMCs/CD4+ T cells isolated from psoriasis patients treated with 720 mg/day DMF – *in vitro* | IFN-γ (Th1) levels | Inhibition | Ghoreschi *et al.* 2011[17] |
| | | | IL-4 (Th2) levels | Stimulation | |
| DMF | 70 µM | DCs (human/mouse bone marrow) and T cell-depleted spleen cells (APCs) | GSH levels | Depletion | |
| | | | IL-12 and IL-23 expression | Inhibition | |
| | | | HO-1 expression | Stimulation | |
| MMF | 1 µg/mL | Murine motor neuron cells – *in vitro* | Caspase-3 expression | Depletion | Linker *et al.* 2011[31] |
| | 0.43–3.9 µg/mL | Human primary spinal cord astrocytes – *in vitro* | Cell death | Inhibition | |
| | 5–50 µM | Cultured human astrocytes – *in vitro* | Nrf2 levels | Stimulation | |
| | | Cultured rodent (mouse and rat) astrocytes – *in vitro* | Nrf2 levels | Stimulation | |
| | 10 µg/mL | Murine motor neuron cells | Caspase-3 expression | No effect | |
| DMF | 5–50 µM | Cultured human astrocytes | Nrf2 levels | Stimulation | |
| | | Cultured rodent (mouse and rat) | Nrf2 levels | Stimulation | |
| | 30 µM | Cultured human astrocytes | NQO-1 expression | Stimulation | |
| | | | AKR1B10 expression | Stimulation | |
| DMF | 10 µg/mL | Isolated human PBMCs from psoriasis patients and healthy donors – *in vitro* | IFN-γ (Th1) expression | Inhibition | Tahvili 2015[18] |
| | | | IL-4 (Th2) expression | Stimulation | |
| | | | IL-17, IL-22, and GM-CSF mRNAs | Inhibition | |

[a]At DMF concentrations ≥12 mM/L, a significant release of lactate dehydrogenase from HaCaT cells was observed after 48 hours, indicating a toxic effect.
[b]Only at subtoxic concentrations (4 µM).
APCs, antigen-presenting cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; HaCaT, human hyperproliferative keratinocytes; HEK, human embryonic kidney cells; HLA-DR, human leukocyte antigen DR; HO-1, heme-oxygenase 1; HUVEC, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule 1; IFN-γ, interferon-gamma; IL, interleukin; Keap-1, kelch-like erythroid cell-derived protein with cap'n'collar homology [ECH]-associated protein 1; Nrf-2, nuclear factor (erythroid-derived 2)-like 2; PBMC, peripheral blood mononuclear cells; Th1, T-helper cells type 1; Th2, T-helper cells type 2; TBF, tumour necrosis factor; VCAM, vascular cell adhesion molecule.
*Only concentrations/dosages which caused stimulation/inhibition are presented.

established a role for DMF in tissue factor expression. Loewe and colleagues observed that DMF can selectively prevent the TNF-induced entry of NF-κB proteins into the nucleus. Furthermore, this effect was selective for NF-κB protein downstream of IκB kinase release, as shuttling of NF-κB/IκB complexes was not affected by DMF[27]. Furthermore, addition of NF-κB inhibitors augmented the anti-inflammatory potential of DMF. DMF-dependent inhibition of nuclear NF-κB translocation in TNF-α-stimulated human endothelial cells was greatly enhanced by blocking NF-κB activation *via* a kinase inhibitor of NF-κB-1 (KINK-1), a small molecule inhibitor of inhibitory κB kinase complex (IKKβ)[28]. These changes resulted in downstream reductions in the expression of endothelial adhesion molecules, including E-selectin, vascular cell adhesion protein 1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) (key factors associated with leucocyte extravasation), such that rolling and adhesion of human lymphocytes on TNF-activated endothelial cells was synergistically reduced in this system[28].

Finally, incubation of neutrophil polymorphonuclear granulocytes (PMN, the first cells to infiltrate psoriatic plaques[29]) with DMF (10–20 μg/mL) has been shown to have inhibitory effects on neutrophil extracellular trap (NET) formation[30]. NETs initiate downstream inflammatory pathways including IL-1β dependent pathways and activation of Th17 cells[31], and NETs have thus been implicated as a key driver of psoriatic pathogenesis. Modulation of NET formation by DMF was glutathione-dependent and established *via* a reduction in reactive oxygen species (ROS). Inhibition of NET formation was not reported following treatment with MMF, and there was only a small reduction in ROS production[30]. DMF inhibition of NET formation may therefore contribute to the beneficial role of DMF in the management of psoriasis.

In summary, DMF appears to have significantly greater inhibitory effects on many aspects of inflammatory processes *in vitro* than other FAEs, and particularly MEF. These effects have been reported consistently in *in vitro* studies with near-physiological concentrations of DMF, whereas results in studies of MMF have been inconsistent. Furthermore, only DMF has been shown to inhibit NET formation, which is proposed to be a key driver of psoriatic pathogenesis.

## Antiproliferative and Cytotoxic Profile of FAEs

Early experiments in the mid-1970s identified a role for MEF in cellular processes. Inhibitory activity of MEF was reported by both Hagedorn *et al*.[2] and Petres *et al*.[3] in the context of DNA synthesis, cell proliferation and protein synthesis. Experiments in human lymphocytes and human PHA-stimulated lymphocytes incubated with varying concentrations of MEF all reported inhibition of these cellular processes with this FAE.

Subsequently, Thio and colleagues also reported dose-dependent inhibitory effects for the FAEs on cell proliferation in cultured keratinocytes. The potency of inhibitory action of different FAEs varied, with DMF showing the highest potency, followed by MEF and MMF[10]. FA reported the lowest inhibitory potential[10]. The antiproliferative effects of the FAEs were attributed to modulation of calcium ion release from intracellular stores into the cytoplasm[10]. Similarly, antiproliferative effects of DMF in cultured hyperproliferative HaCaT keratinocytes have been reported[5]; 50% inhibition concentrations ($IC_{50}$) for DNA/protein synthesis were 2.3/2.5 μmol/L for DMF, 133/145 μmol/L, 215/230 μmol/L and 275/270 μmol/L for zinc, calcium and magnesium salts of MEF, respectively, and >960 μmol/L for FA[5]. A similar cytotoxic potency profile was observed following incubation of FAEs (at concentrations ranging from 3–100 μmol/L) with lympho-histiocytic U-937 cells. DMF showed the largest dose-dependent apoptotic effect, followed by the zinc and calcium salts of MEF. No apoptotic activity was observed with FA and the magnesium salt of MEF at concentrations <100 μmol/L[12].

In summary, the most active FAE in terms of *in vitro* antiproliferative and cytotoxic effects is DMF. $IC_{50}$s for the effects of DMF *in vitro* are in the same range as the serum concentrations observed in clinical studies, which is not the case for other FAEs.

## FAE Effects on Antioxidant and Neuroprotective Pathways

The FAEs can activate the Nrf-2 pathway, which is considered to represent an endogenous defence mechanism against oxidative stress[8,32,33]. Under physiological conditions, Nrf-2 is sequestered in the cytoplasm by the kelch-like ECH-associated protein 1 (KEAP1) so that it may be targeted for ubiquitation and subsequent proteasomal degradation[34,35]. Under conditions of oxidative stress, or in the presence of electrophiles, an allosteric conformational change in KEAP1 cysteine residues diminishes Nrf-2 degradation such that it can translocate into the nucleus and regulate cytoprotective genes associated with an antioxidant response[36,37]. *In vitro* studies by Brennan and colleagues found that treatment of human embryonic kidney 293 cells with DMF (exhibiting electrophilic activity) modified KEAP1 cysteine residues, while such changes following treatment with MEF were significantly smaller and/or undetectable[8]. Modification of KEAP1 with DMF treatment was associated with nuclear translocation of Nrf-2 and a downstream transcriptional response in

treated cells. As before, these effects occurred to a lesser extent on incubation with MEF[8]. Acute concentration-dependent depletion of GSH was also reported with DMF treatment; however, levels recovered above baseline within 24 hours. GSH reduction was not observed with MEF, and increased GSH levels above baseline were still observed at 24 hours[8]. More recently, work by Helwa and colleagues has reported that MMF can stimulate Nrf-2 and aquaporin-3 (AQP3) expression in primary mouse keratinocytes and modulate downstream keratinocyte functionality[16]. Previous studies have found evidence for a role of AQP3 in keratinocyte differentiation[38,39] and may offer a means by which the effects of MMF on keratinocytes are mediated[16].

Neuroprotective effects of the FAEs on glial cells and neurons[32], and suppression of IL-12 and IL-23 production by dendritic cells[40], have also been documented. Application of DMF to murine neuronal cells *in vitro* has been found to enhance survival and protect rodent or human astrocyte cells from oxidative stress *via* activation of the Nrf-2 pathway[32]. Similarly, like DMF, sulforaphane (SFN) is an immune-modulating compound derived from natural products and has been shown to suppress expression of IL-23 and IL-12 *in vivo* and augmented Th17- and Th1-mediated responses within the central nervous system[40].

Both DMF and MMF appear to have antioxidant effects *in vitro*, while MEF does not. DMF also has neuroprotective effects at physiological concentrations.

## FAE Effects on Cell-Adhesion Molecules

Other actions of DMF that have been reported in the literature (Table 1), include modulation of ICAM-1, E-selectin and VCAM-1 expression[4,7,11,14]. In human umbilical vein endothelial cells (HUVECs), Vandermeeren and colleagues reported reduced expression of ICAM-1, VCAM-1 and E-selectin following incubation with DMF. In contrast, MEF and FA had no effect on expression of these molecules[7]. In another study, incubation of HUVECs and human lymphocytes with DMF, but not with MMF, also resulted in inhibition of expression of ICAM-1, VCAM-1 and E-selection. This study also reported inhibition of leucocyte/endothelial cell interactions such as cell rolling and adhesion[14]. Finally, IFNγ-induced expression of ICAM-1 and human leucocyte antigen-DR (HLA-DR) on hyperproliferative HaCaT keratinocytes was suppressed with subtoxic concentrations of DMF and provides further support for the role of DMF in modulation of cell adhesion[11]. As described previously, regulation of NF-κB signalling by DMF in endothelial cells has been shown to have downstream consequences for cell-adhesion molecule signalling expression[28].

## FAE Effects on Angiogenesis

It has also been postulated that FAEs may have an anti-angiogenic component to their functionality[41–43]. DMF was found to decrease tube formation in human endothelial cells *in vitro*. Cells treated with DMF had decreased expression of vascular endothelial growth factor receptor-2 (VEGFR-2), but not VEGFR-1 or neuropilin-1[43]. Other investigators also demonstrated tube formation inhibition with DMF but not with MMF or FA[42]. DMF also did not inhibit the kinase activity of VEGFR-2, and anti-angiogenic activity was demonstrated in two *in vivo* models[42]. DMF was shown in these models (chick chorioallantoic membrane and live fluorescent zebrafish embryo neovascularization assays) to attenuate the differentiation, proliferation and migration of endothelial cells and in doing so to block angiogenesis pathways. As observed previously, these effects were not replicated with MEF or FA. Inhibition of growth in transformed and untransformed cells by DMF has been postulated to occur *via* induction of apoptosis[42].

## Conclusions

Overall, *in vitro* evidence to date indicates that MEF salts have less biological activity than DMF and MMF[5,13,14,21,44,45], as well as differing pharmacodynamic characteristics compared with DMF[46] (Table 1). The *in vitro* data justify the hypothesis that DMF is the key ingredient of Fumaderm® and mainly or even exclusively responsible for its antipsoriatic activity. This conclusion is supported by the results of animal experiments, which we have reviewed recently (Landeck *et al.*, submitted). Most importantly, however, this conclusion has been proven by a double-blind, randomized, placebo-controlled phase III clinical study demonstrating that DMF is as effective as Fumaderm® for the treatment of moderate-to-severe psoriasis[47].

## Conflict of Interest

LL has no conflicts of interest to declare. AA and IPC are employees of Almirall, S.A. KA has served as consultant, advisory board member, or speaker for AbbVie, Antabio, Almirall, EmertiPharma, Galderma, Leo, Loreal, Eli Lilly, and Novartis.

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## References

1. Nimmesgern E, Benediktsson I, Norstedt I, 2017, Personalized medicine in Europe. *Clinical and Translational Science*,

vol.10(2): 61–63.
https://dx.doi.org/10.1111/cts.12446

2. Hagedorn M, Kalkoff K W, Kiefer G, *et al.*, 1975, *Fumarsäuremonoäthylester: Wirkung auf DNA-synthese und erste tierexperimentelle befunde* (German) [Fumaric acid monoethylester: Effect on DNA-synthesis and preliminary findings in experimental studies in animals]. *Archives of Dermatological Research*, vol.254(1): 67–73.
https://dx.doi.org/10.1007/BF00561536
3. Petres J, Kalkoff K W, Baron D, *et al.*, 1975, *Der Einfluss von Fumarsäuremonoäthylester auf die Nucleinsäure- und Proteinsynthese PHA-stimulierter menschlicher Lymphocyten* (German) [The effect of fumaric acid monoethylester on the synthesis of nucleic acids and proteins of PHA-stimulated human lymphocytes]. *Archiv fur Dermatologische Forschung*, vol.251(4): 295–300.
https://dx.doi.org/10.1007/BF00562233
4. Nibbering P H, Thio B, Zomerdijk T P, *et al.*, 1993, Effects of monomethylfumarate on human granulocytes. *Journal of Investigative Dermatology*, vol.101(1): 37–42.
https://dx.doi.org/10.1111/1523-1747.ep12358715
5. Sebok B, Bonnekoh B, Geisel J, *et al.*,1994, Antiproliferative and cytotoxic profiles of antipsoriatic fumaric acid derivatives in keratinocyte cultures. *European Journal of Pharmacology*, vol.270(1): 79–87.
https://dx.doi.org/10.1016/0926-6917(94)90083-3
6. de Jong R, Bezemer A C, Zomerdijk T P, *et al.*,1996, Selective stimulation of T helper 2 cytokine responses by the anti-psoriasis agent monomethylfumarate. *European Journal of Immunology*, vol.26(9): 2067–2074.
https://dx.doi.org/10.1002/eji.1830260916
7. Vandermeeren M, Janssens S, Borgers M, *et al.*, 1997, Dimethylfumarate is an inhibitor of cytokine-induced E-selectin, VCAM-1, and ICAM-1 expression in human endothelial cells. *Biochemical and Biophysical Research Communications*, vol.234(1): 19–23.
https://dx.doi.org/10.1006/bbrc.1997.6570
8. Brennan M S, Matos M F, Li B, *et al.*, 2015, Dimethyl fumarate and monoethylfumarate exhibit differential effects on KEAP1, NRF2 activation, and glutathione depletion *in vitro*. *PLoS One*, vol.10(3): e0120254.
https://dx.doi.org/10.1371/journal.pone.0120254
9. Gillard G O, Collette B, Anderson J, *et al.*, 2015, DMF, but not other fumarates, inhibits NF-kappaB activity *in vitro* in an Nrf2-independent manner. *Journal of Neuroimmunology*, vol.283: 74–85.
https://dx.doi.org/10.1016/j.jneuroim.2015.04.006
10. Thio H B, Zomerdijk T P, Oudshoorn C, *et al.*, 1994, Fumaric acid derivatives evoke a transient increase in intracellular free calcium concentration and inhibit the proliferation of human keratinocytes. *British Journal of Dermatology*, vol.131(6): 856–861.
https://dx.doi.org/10.1111/j.1365-2133.1994.tb08589.x
11. Sebok B, Bonnekoh B, Vetter R, *et al.*, 1998, The antipsoriatic dimethyl-fumarate suppresses interferon-gamma-induced ICAM-1 and HLA-DR expression on hyperproliferative keratinocytes. Quantification by a culture plate-directed APAAP-ELISA technique. *European Journal of Dermatology*, vol.8(1): 29–32.
12. Sebök B, Gollnick H, Mahrle G, *et al.*, 2000, Dimethyl-fumarate is the Fumaderm-compound with the strongest induction of apoptosis-phenomena in lympho-histiocytic U-937 cells. *Zeitschrift fur Hautkrankheiten*, vol.76: 347–351.
13. Lehmann J C, Listopad J J, Rentzsch C U, *et al.*, 2007, Dimethylfumarate induces immunosuppression *via* glutathione depletion and subsequent induction of heme oxygenase 1. *Journal of Investigative Dermatology*, vol.127(4): 835–845.
https://dx.doi.org/10.1038/sj.jid.5700686
14. Wallbrecht K, Drick N, Hund A C, *et al.*, 2011, Downregulation of endothelial adhesion molecules by dimethylfumarate, but not monomethylfumarate, and impairment of dynamic lymphocyte-endothelial cell interactions. *Experimental Dermatology*, vol.20(12): 980–985.
https://dx.doi.org/10.1111/j.1600-0625.2011.01376.x
15. McGuire V A, Ruiz-Zorrilla Diez T, Emmerich C H, *et al.*, 2016, Dimethyl fumarate blocks pro-inflammatory cytokine production *via* inhibition of TLR induced M1 and K63 ubiquitin chain formation. *Scientific Reports*, vol.6(31159): 31159.
https://dx.doi.org/10.1038/srep31159
16. Helwa I, Choudhary V, Chen X, *et al.*, 2017, The anti-psoriatic drug monomethylfumarate increases nuclear factor erythroid 2-related factor 2 (Nrf2) levels and induces aquaporin-3 (AQP3) mRNA and protein expression. *Journal of Pharmacology and Experimental Therapeutics*,vol.362(2): 243–253.
https://dx.doi.org/10.1124/jpet.116.239715
17. Ghoreschi K, Bruck J, Kellerer C, *et al.*, 2011, Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *Journal of Experimental Medicine*, vol.208(11): 2291–2303.
https://dx.doi.org/10.1084/jem.20100977

18. Tahvili S, Zandieh B, Amirghofran Z, 2015, The effect of dimethyl fumarate on gene expression and the level of cytokines related to different T helper cell subsets in peripheral blood mononuclear cells of patients with psoriasis. *International Journal of Dermatology*, vol.54(7): e254–260. https://dx.doi.org/10.1111/ijd.12834
19. Rostami-Yazdi M, Clement B, Mrowietz U, 2010, Pharmacokinetics of anti-psoriatic fumaric acid esters in psoriasis patients. *Archives of Dermatological Research*, vol.302(7): 531–538. https://dx.doi.org/10.1007/s00403-010-1061-4
20. Mrowietz U, Christophers E, Altmeyer P, 1999, Treatment of severe psoriasis with fumaric acid esters: Scientific background and guidelines for therapeutic use. *British Journal of Dermatology*, vol.141(3): 424–429. https://dx.doi.org/10.1046/j.1365-2133.1999.03034.x
21. Ockenfels H M, Schultewolter T, Ockenfels G, *et al.*, 1998, The antipsoriatic agent dimethylfumarate immunomodulates T-cell cytokine secretion and inhibits cytokines of the psoriatic cytokine network. *British Journal of Dermatology*, vol.139(3): 390–395. https://dx.doi.org/10.1046/j.1365-2133.1998.02400.x
22. Nibbering P H, Thio B, Bezemer A C, *et al.*, 1997, Intracellular signalling by binding sites for the antipsoriatic agent monomethylfumarate on human granulocytes. *British Journal of Dermatology*, vol.137(1): 65–75. https://dx.doi.org/10.1046/j.1365-2133.1997.1769185.x
23. Asadullah K, Schmid H, Friedrich M, *et al.*, 1997, Influence of monomethylfumarate on monocytic cytokine formation-explanation for adverse and therapeutic effects in psoriasis? *Archives of Dermatological Research*, vol.289(11): 623–630. https://dx.doi.org/10.1007/s004030050251
24. Gillard G, Huss D, Anderson J, *et al.*, 2014, Fumarate esters are distinguished by differential inhibition of the NF-κB mediated proinflammatory response. *Minerva Medica*, vol.52(2): 428
25. Menter A, Korman N J, Elmets C A, *et al.*, 2009, Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 3. Guidelines of care for the management and treatment of psoriasis with topical therapies. *Journal of the American Academy of Dermatology*, vol.60(4): 643–659. https://dx.doi.org/10.1016/j.jaad.2009.03.027
26. Hoxtermann S, Nuchel C, Altmeyer P, 1998, Fumaric acid esters suppress peripheral CD4- and CD8-positive lymphocytes in psoriasis. *Dermatology*, vol.196(2): 223–230. https://dx.doi.org/10.1159/000017903
27. Loewe R, Holnthoner W, Groger M, *et al.*, 2002, Dimethylfumarate inhibits TNF-induced nuclear entry of NF-kappa B/p65 in human endothelial cells. *Journal of Immunology*, vol.168(9): 4781–4787. https://dx.doi.org/10.4049/jimmunol.168.9.4781
28. Hund A C, Lockmann A, Schon M P, 2016, Mutually enhancing anti-inflammatory activities of dimethyl fumarate and NF-kappaB inhibitors-implications for dose-sparing combination therapies. *Experimental Dermatology*, vol.25(2): 124–130. https://dx.doi.org/10.1111/exd.12892
29. Christophers E, Metzler G, Rocken M, 2014, Bimodal immune activation in psoriasis. *British Journal of Dermatology*, vol.170(1): 59–65. https://dx.doi.org/10.1111/bjd.12631
30. Hoffmann JHO, Schaekel K, Hartl D, *et al.*, 2017, Dimethyl fumarate modulates neutrophil extracellular trap formation in a glutathione and superoxide-dependent manner. *British Journal of Dermatology*. https://dx.doi.org/10.1111/bjd.15839
31. Warnatsch A, Ioannou M, Wang Q, *et al.*, 2015, Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science*, vol.349(6245): 316–320. https://dx.doi.org/10.1126/science.aaa8064
32. Linker R A, Lee D H, Ryan S, *et al.*, 2011, Fumaric acid esters exert neuroprotective effects in neuroinflammation *via* activation of the Nrf2 antioxidant pathway. *Brain*, vol.134(Pt 3): 678–692. https://dx.doi.org/10.1093/brain/awq386
33. Scannevin R H, Chollate S, Jung M Y, *et al.*, 2012, Fumarates promote cytoprotection of central nervous system cells against oxidative stress *via* the nuclear factor (erythroid-derived 2)-like 2 pathway. *Journal of Pharmacology and Experimental Therapeutics*, vol.341(1): 274–284. https://dx.doi.org/10.1124/jpet.111.190132
34. Itoh K, Wakabayashi N, Katoh Y, *et al.*, 2003, Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. *Journal of Genes to Cells*, vol.8(4): 379–391. https://dx.doi.org/10.1046/j.1365-2443.2003.00640.x
35. Kobayashi A, Kang M I, Okawa H, *et al.*, 2004, Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Molecular and Cellular Biology*, vol.24(16): 7130–7139. https://dx.doi.org/10.1128/MCB.24.16.7130-7139.2004

36. Itoh K, Chiba T, Takahashi S, *et al.*, 1997, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochemical and Biophysical Research Communications*, vol.236(2): 313–322. https://dx.doi.org/10.1006/bbrc.1997.6943
37. Nguyen T, Sherratt PJ, Pickett C B, 2003, Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annual Review of Pharmacology and Toxicology*, vol.43: 233–260. https://dx.doi.org/10.1146/annurev.pharmtox.43.100901.140229
38. Bollag W B, Xie D, Zheng X, *et al.*, 2007, A potential role for the phospholipase D2-aquaporin-3 signaling module in early keratinocyte differentiation: production of a phosphatidylglycerol signaling lipid. *Journal of Investigative Dermatology*, vol.127(12): 2823–2831. https://dx.doi.org/10.1038/sj.jid.5700921
39. Choudhary V, Olala L O, Qin H, *et al.*, 2015, Aquaporin-3 re-expression induces differentiation in a phospholipase D2-dependent manner in aquaporin-3-knockout mouse keratinocytes. *Journal of Investigative Dermatology*, vol.135(2): 499–507. https://dx.doi.org/10.1038/jid.2014.412
40. Geisel J, Bruck J, Glocova I, *et al.*, 2014, Sulforaphane protects from T cell-mediated autoimmune disease by inhibition of IL-23 and IL-12 in dendritic cells. *Journal of Immunology*, vol.192(8): 3530–3539. https://dx.doi.org/10.4049/jimmunol.1300556
41. Arbiser J L, 2011, Fumarate esters as angiogenesis inhibitors: Key to action in psoriasis. *Journal of Investigative Dermatology*, vol.131(6): 1189–1191. https://dx.doi.org/10.1038/jid.2011.45
42. Garcia-Caballero M, Mari-Beffa M, Medina MA, *et al.*, 2011, Dimethylfumarate inhibits angiogenesis *in vitro* and *in vivo*: A possible role for its antipsoriatic effect? *Journal of Investigative Dermatology*, vol.131(6): 1347–1355. https://dx.doi.org/10.1038/jid.2010.416
43. Meissner M, Doll M, Hrgovic I, *et al.*, 2011, Suppression of VEGFR2 expression in human endothelial cells by dimethylfumarate treatment: Evidence for anti-angiogenic action. *Journal of Investigative Dermatology*, vol.131(6): 1356–1364. https://dx.doi.org/10.1038/jid.2011.46
44. Treumer F, Zhu K, Glaser R, *et al.*, 2003, Dimethylfumarate is a potent inducer of apoptosis in human T cells. *Journal of Investigative Dermatology*, vol.121(6): 1383–1388. https://dx.doi.org/10.1111/j.1523-1747.2003.12605.x
45. Rubant S A, Ludwig R J, Diehl S, *et al.*, 2008, Dimethylfumarate reduces leukocyte rolling *in vivo* through modulation of adhesion molecule expression. *Journal of Investigative Dermatology*, vol.128(2): 326–331. https://dx.doi.org/10.1038/sj.jid.5700996
46. Brennan M, Allaire N, Huss D, *et al.*, 2014, Dimethyl fumarate and monomethyl fumarate are distinguished by non-overlapping pharmacodynamic effects *in vivo*. *Neurology*, vol.82: P1.206.
47. Mrowietz U, Szepietowski J C, Loewe R, *et al.*, 2017, Efficacy and safety of LAS41008 (dimethyl fumarate) in adults with moderate-to-severe chronic plaque psoriasis: A randomized, double-blind, Fumaderm(R)- and placebo-controlled trial (BRIDGE). *British Journal of Dermatology*, vol.176(3): 615–623. https://dx.doi.org/10.1111/bjd.14947