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

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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

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 10–15 μmol/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 cocultured 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[21].

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[4].

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>
<thead>
<tr>
<th>FAE compound</th>
<th>Target cells</th>
<th>Cellular activities</th>
<th>Inhibition/stimulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MEF</strong></td>
<td>0.1–500 µg/mL</td>
<td>Cultured PHA-simulated human lymphocytes</td>
<td>Nucleic acid synthesis</td>
<td>Inhibition</td>
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<td></td>
<td></td>
<td></td>
<td>Protein synthesis</td>
<td>Inhibition</td>
</tr>
<tr>
<td><strong>DMF</strong></td>
<td>0.4–960 mM/L</td>
<td>Human HaCaT cell line – <em>in vitro</em></td>
<td>Proliferation</td>
<td>Inhibition</td>
</tr>
<tr>
<td></td>
<td>5–30 µM/L</td>
<td>Co-cultured human keratinocytes and lymphocytes</td>
<td>IL-10</td>
<td>Stimulation</td>
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<td>TGF-α</td>
<td>Inhibition</td>
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<td>IFNγ</td>
<td>Inhibition</td>
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<tr>
<td><strong>MMF</strong></td>
<td>200 mM/L</td>
<td>Granulocytes from healthy donors – <em>in vitro</em></td>
<td>Respiratory burst</td>
<td>Inhibition</td>
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<td></td>
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<td>DNA and protein synthesis</td>
<td>Inhibition</td>
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<tr>
<td><strong>MEF</strong></td>
<td>0.4–960 mM/L</td>
<td>MEF-Zn HaCaT cell line</td>
<td>DNA and protein synthesis</td>
<td>Inhibition</td>
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<td>MEF-Ca</td>
<td>Inhibition</td>
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<td>MEF-Mg</td>
<td>Inhibition</td>
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<td>No effect</td>
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<tr>
<td><strong>MMF</strong></td>
<td>0.05–0.4 mM</td>
<td>Human juvenile keratinocytes (normal and SV-40-transformed) – <em>in vitro</em></td>
<td>Intracellular free Ca release</td>
<td>Stimulation</td>
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<td>Proliferation</td>
<td>Inhibition</td>
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<td></td>
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<td>Proliferation</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

[^3]: Reports of *in vitro* concentrations
[^4]: Reports of *in vitro* dosages
[^7]: Petres et al. 1975
[^8]: Sebők et al. 1994
[^9]: De Jong et al. 1996
[^21]: Ockenfels et al. 1998
### Table 1. *In vitro* and *in vivo* effects of MEF, DMF and MMF on different target cells (Continued)

<table>
<thead>
<tr>
<th>FAE compound</th>
<th>Target cells</th>
<th>Cellular activities</th>
<th>Inhibition/stimulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMF</strong> 100 µM</td>
<td>Cultured human PBMCs from psoriatic patients and healthy volunteers – <em>in vitro</em></td>
<td>IL-10, TNF-α, IL-1RA</td>
<td>Stimulation, Stimulation, Stimulation</td>
<td>Asadullah et al. 1997[^22]</td>
</tr>
<tr>
<td><strong>DMF</strong> 1-319 µM</td>
<td>HaCaT cell line and normal human epidermal keratinocytes – <em>in vitro</em></td>
<td>Expression of ICAM and HLA-DR</td>
<td>Inhibition</td>
<td>Sebök et al. 1998[^11]</td>
</tr>
<tr>
<td><strong>DMF</strong> 3–100 µM</td>
<td>Human lympho-histiocytic cell line (U937) – <em>in vitro</em></td>
<td>Apoptosis</td>
<td>Stimulation</td>
<td>Sebök et al. 2000[^2]</td>
</tr>
<tr>
<td><strong>MEF</strong> 3–100 µM MEF-Zn</td>
<td>U937 lympho-histiocytic cell line</td>
<td>Apoptosis</td>
<td>Stimulation</td>
<td>Sebök et al. 2000[^2]</td>
</tr>
<tr>
<td><strong>DMF</strong> 1–100 µM</td>
<td>Cultured human lymphocytes – <em>in vitro</em></td>
<td>Proliferation</td>
<td>Inhibition</td>
<td>Lehmann et al. 2007[^13]</td>
</tr>
<tr>
<td><strong>MMF</strong> 1–100 µM</td>
<td>Cultured human lymphocytes – <em>in vitro</em></td>
<td>Proliferation, Cytokine production (TNFα)</td>
<td>No effect</td>
<td>Lehmann et al. 2007[^13]</td>
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<td>No effect</td>
<td>Lehmann et al. 2007[^13]</td>
</tr>
<tr>
<td><strong>DMF</strong> 5–200 µM</td>
<td>Human placental microvascular endothelial cells (HPEC-A2)/normal human lymphocytes – <em>in vitro</em></td>
<td>Expression of ICAM, VCAM and E-selectin</td>
<td>Inhibition</td>
<td>Wallbrecht et al. 2011[^10]</td>
</tr>
<tr>
<td><strong>MMF</strong> 5–200 µM</td>
<td>Human placental microvascular endothelial cells (HPEC-A2)/normal human lymphocytes – <em>in vitro</em></td>
<td>Expression of ICAM, VCAM and E-selectin</td>
<td>No effect</td>
<td>Wallbrecht et al. 2011[^10]</td>
</tr>
<tr>
<td><strong>DMF</strong> 25–100 µM</td>
<td>Bone marrow-derived murine macrophages – <em>in vitro</em></td>
<td>Cytokine induction and gene transcription (Inc. TNF, IL-6, IL-10, GM-CSF)</td>
<td>Inhibition</td>
<td>McGuire 2016[^15]</td>
</tr>
<tr>
<td><strong>MMF</strong> 0–750 µM</td>
<td>Cultured primary murine keratinocytes</td>
<td>Nrf-2 expression, HO-1 expression, AQP3 expression</td>
<td>Stimulation, Stimulation, Stimulation</td>
<td>Helwa et al. 2017[^28]</td>
</tr>
<tr>
<td><strong>DMF</strong> 10–20 mg/L</td>
<td>Human polymorphonuclear granulocytes from psoriasis patients – <em>in vitro</em></td>
<td>NET expression, ROS expression, GSH levels</td>
<td>Inhibition, Depletion, Depletion</td>
<td>Hoffmann et al. 2017[^29]</td>
</tr>
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</table>

Reports investigating *in vitro* dosage and effect of *in vivo* dosing*:

<table>
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<td><strong>MEF</strong> 0.1–500 µg/mL</td>
<td>Cultured human lymphocytes</td>
<td>DNA synthesis, Proliferation</td>
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<tr>
<td>DMF</td>
<td>PBMCs/CD4+ T cells isolated from psoriasis patients treated with 720 mg/day DMF – in vitro</td>
<td>IFN-γ (Th1) levels, IL-4 (Th2) levels</td>
<td>Inhibition, Stimulation</td>
<td>Ghoreschi et al. 2011[17]</td>
</tr>
</tbody>
</table>
| DMF          | DCs (human/mouse bone marrow) and T cell-depleted spleen cells (APCs) | GSH levels, IL-12 and IL-23 expression, HO-1 expression | Depletion, Inhibition, Stimulation | |}
| MMF          | Murine motor neuron cells – in vitro | Caspase-3 expression, Cell death | Depletion, Inhibition | Linker et al. 2011[31] |
|              | Human primary spinal cord astrocytes – in vitro | Nrf2 levels | Stimulation | |}
|              | Cultured human astrocytes – in vitro | Nrf2 levels | Stimulation | |}
|              | Cultured rodent (mouse and rat) astrocytes – in vitro | Nrf2 levels | Stimulation | |}
| DMF          | Murine motor neuron cells | Caspase-3 expression | No effect | |}
| DMF          | Cultured human astrocytes | Nrf2 levels | Stimulation | |}
| DMF          | Cultured rodent (mouse and rat) | Nrf2 levels | Stimulation | |}
| DMF          | Cultured human astrocytes | NQO-1 expression, AKR1B10 expression | Stimulation | |}
| DMF          | Isolated human PBMCs from psoriasis patients and healthy donors – in vitro | IFN-γ (Th1) expression, IL-4 (Th2) expression, IL-17, IL-22, and GM-CSF mRNAs | Inhibition, Stimulation | Tahvili 2015[18] |

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[25]. 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/IκB complexes (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[29].

Finally, incubation of neutrophil polymorphonuclear granulocytes (PMN, the first cells to infiltrate psoriatic plaques[26]) 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[9] 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[2]; 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} 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 Neurproective 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 ubiquitination[10,25]. A similar cytotoxic potency profile was observed following incubation of FA reported the lowest inhibitory potential[5]. 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[10,13]. 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 [9]. 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 [10]. Previous studies have found evidence for a role of AQP3 in keratinocyte differentiation [10,19] and may offer a means by which the effects of MMF on keratinocytes are mediated [10].

Neuroprotective effects of the FAEs on glial cells and neurons [32], and suppression of IL-12 and IL-23 production by dendritic cells [46], have also been documented. Application of DMF to murine neuronal cells in vitro has been found to enhance survival and protect rodent or human astrocytes 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 vitro. 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 [3,7,11,14]. 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 [25].

**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, EmeritiPharma, Galderma, Leo, Loreal, Eli Lilly, and Novartis.

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

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