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# Oral fumaric acid esters for the treatment of active multiple sclerosis: an open-label, baseline-controlled pilot study

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An exploratory, prospective, open-label study of fumaric acid esters (FAE, Fumaderm®) was conducted in patients with relapsing–remitting multiple sclerosis (RRMS). The study consisted of the following four phases: 6-week baseline, 18-week treatment (target dose of 720 mg/day), 4-week washout, and a second 48-week treatment phase (target dose of 360 mg/day). Ten patients with an Expanded Disability Status Scale (EDSS) score of 2.0–6.0 and at least one gadolinium-enhancing (Gd+) lesion on T1-weighted magnetic resonance imaging (MRI) brain scans participated in the study. Safety was assessed by adverse events (AEs), blood chemistry/hematology, electrocardiogram, and urinalysis. The primary efficacy outcomes were number and volume of Gd+ lesions. Other clinical outcomes included EDSS score, ambulation index (AI), and nine-hole peg test (9-HPT). Effects of FAE on intracellular cytokine profiles, T-cell apoptosis, and soluble adhesion molecules were also assessed. Three patients withdrew during the first 3 weeks of the study because of side effects, non-compliance, and follow-up loss. The most common AEs were gastrointestinal symptoms and flushing; all AEs were reported as mild and reversible. FAE produced significant reductions from baseline in number ($P < 0.05$) and volume ($P < 0.01$) of Gd+ lesions after 18 weeks of treatment; this effect persisted during the second treatment phase at half the target dose after the 4-week washout period. EDSS scores, AI, and 9-HPT remained stable or slightly improved from baseline in all patients. Measures of T-cell function demonstrated alterations in cytokines and circulating tumor necrosis factor. The results of this exploratory study suggest that further studies of FAE in patients with MS are warranted.

## Introduction

Multiple sclerosis (MS), a major cause of chronic disability in young adults, is pathologically characterized by focal areas of demyelination and axonal loss in the central nervous system (CNS). Although the etiology of MS is uncertain, several lines of evidence indicate that autoimmune response plays a central role in the development of MS lesions. First, myelin breakdown products have been detected in macrophages in MS lesions and in the cerebrospinal fluid (CSF) of MS patients [1,2]. Secondly, MS lesions have many features of a delayed-type hypersensitivity reaction [3], and demonstrate the following: increased levels of lymphokines and cytokines [interferon (IFN)-$\gamma$, tumor necrosis factor (TNF)-$\alpha$, interleukin (IL)-1]; activated $CD4^+$ and $CD8^+$ T cells; mononuclear phagocytes (macrophages, microglia, and monocytes) expressing variable levels of class II major histocompatibility complex (MHC) antigens; and upregulation of leukocyte and vascular adhesion molecules (VCAM)-1 [4–7]. Thirdly, CSF of MS patients shows intrathecal synthesis of immunoglobulins with restricted heterogeneity [8] and an increased frequency of autoreactive T cells that secrete IFN-$\gamma$ in response to myelin proteins [9]. Fourthly, agents with immunomodulatory properties, such as IFN$\beta$ have been shown to reduce lesion formation, decrease the frequency of relapses, and slow the progression of disability in MS [10–12].

Fumaric acid esters (FAE) influence several aspects of immune functions that are thought to be involved in MS. FAE therapy has been shown to induce T-helper (Th)2-like cytokines (e.g. IL-4, IL-5, and IL-10) [13,14] to induce apoptosis in activated T cells [13] and to downregulate intracellular adhesion molecules (ICAM)-1 and VCAM expression [15]. A reduction in these cellular adhesion molecules may lead to reduced

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migration of lymphocytes across endothelial barriers into surrounding tissues, an important event in MS [6]. Fumaderm® (Fumapharm, Muri, Switzerland), a FAE formulation, is approved in Germany for the treatment of severe chronic plaque psoriasis [16–21]. The efficacy and safety of oral FAE (Fumaderm®) were investigated in a baseline-controlled, open-label pilot study of patients with relapsing–remitting MS (RRMS).

## Methods

### Patients

Ten patients were enrolled in the study. Patients were eligible for enrollment if they were 18–55 years of age, had a definite diagnosis of RRMS [22], and had at least one relapse within the year prior to enrollment. In addition, patients had at least one active lesion on magnetic resonance imaging (MRI) brain scans and a baseline Expanded Disability Status Scale (EDSS) score of 2.0–6.0. Patients were excluded from the study if they had any of the following: an infection, a chronic inflammatory disease other than MS, a history of drug or alcohol abuse, a disease exacerbation or corticosteroid treatment within 30 days, or immunosuppressive or immunomodulatory therapy within 12 weeks of enrollment. Patients who were pregnant or breastfeeding were also excluded. The study protocol was approved by the local ethics committee, and all patients were counseled regarding current treatment guidelines for MS, including the use of IFN$\beta$ and glatiramer acetate.

### Study design and assessments

This was a prospective, open-label, baseline-controlled exploratory study conducted at St Josef Hospital at Ruhr University in Bochum, Germany. Patients were followed for a total of 70 weeks. The study was composed of the following four phases: a 6-week baseline phase (weeks −6 to 0), an 18-week treatment phase (weeks 0–18), a 4-week washout (no treatment) phase (weeks 19–22), followed by a second 48-week treatment phase (weeks 23–70). At the beginning of each treatment phase, the FAE dose was slowly up-titrated over 9 weeks to minimize gastrointestinal side effects. The maximum daily number of tablets of FAE administered was six per day in the first treatment phase (720 mg/day) and three per day (360 mg/day) in the second treatment phase.

Safety was assessed by the incidence and severity of adverse events, physical and neurologic examinations, blood chemistry/hematology, electrocardiogram (ECG), and urinalysis. The primary efficacy outcomes were the number and volume of gadolinium-enhancing (Gd+) lesions. Clinical outcomes included EDSS score, ambulation index (AI), and nine-hole peg test (9-HPT). The effects of FAE on intracellular cytokine profiles, T-cell apoptosis, and soluble adhesion molecules were also assessed. A physical examination, EDSS, AI, and 9-HPT were performed at screening, baseline, and at weeks 3, 6, 12, 18, 22, 46, and 70. MRI brain scans were performed at screening, baseline, and at weeks 12, 18, 22, 46, and 70. Adverse events were recorded throughout the study. Serum chemistries, ECG, and urinalysis were performed at screening, baseline, and at weeks 1, 3, 6, 9, 12, 18, 22, 28, 34, 40, 46, 52, 58, 64, and 70.

### Study drug

Fumaric acid ester tablets (Fumaderm®, Fumapharm, Muri, Switzerland) were composed of the following: ethylhydrogenfumarate-Ca salt 67 mg, ethylhydrogenfumarate-Mg salt 5 mg, dimethylfumarate 30 mg, ethylhydrogenfumarate-Zn salt 3 mg (Fumaderm initial®); and dimethylfumarate 120 mg, ethylhydrogenfumarate-Ca salt 87 mg, ethylhydrogenfumarate-Mg salt 5 mg, ethylhydrogenfumarate-Zn salt 3 mg (Fumaderm forte®).

### MRI protocol

All MRI scans were performed using a 2-Tesla Bruker Tomikon S200 scanner (Bruker Medizintechnik GmbH, Ettlingen, Germany). Initial T1-weighted (time of repetition (TR) = 800 ms, time of echo (TE) = 17 ms) brain scans, 3-mm-thick axial slices with a spatial resolution of 0.98 mm, were acquired. Each scan was composed of 44 contiguous interwoven images. Triple dose (0.3 mmol/kg body weight) Gd-diethylenetriamine pentaacetic acid (Gd-DPTA, Magnevist®, Schering AG, Berlin, Germany) was administered for higher lesion detection sensitivity [23]. After a 7-min delay, Gd+ T1-weighted scans were then performed using the same parameters as for the native scan. Image data were converted to an 8-bit grayscale tiff-format, providing maximum available contrast. Data analysis was performed using customized Image Tool® scripts for semi-automated image data utilization (UTHSCSA *Image Tool* program, University of Texas Health Science Center, San Antonio, TX, USA; http://ddsdx.uthscsa.edu/dig/itdesc.html). Two independent radiologists detected and counted Gd+ lesions by comparing native T1 images to post-contrast T1 images. The radiologists involved in these assessments were blinded to the treatment status of the patients. To determine the approximate volume of each Gd+ lesion, a semi-automated local threshold procedure counted enhancing pixels to within a maximum of 1/e-of-

maximum contrast to non-enhancing pixels of the lesion environs. Questionable cases were discussed with a third senior radiologist who was also blinded with regard to the treatment status of patients in the study.

### Cytokine expression and apoptotic rate

The intracellular expression of IL-2, IL-4, IL-10, IFN-$\gamma$, TNF-$\alpha$, and transforming growth factor (TGF)-$\beta$ was examined in the $CD4^+$ lymphocyte population [24,25]. Total T lymphocytes were prepared for flow cytometry and stained with anti-CD4 antibodies plus antibodies against one of the cytokines of interest or Annexin V as a marker for apoptosis. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from venous blood samples collected in heparinized tubes using a FICOLL gradient (FICOLLpaque, Pharmacia, Uppsala, Sweden). Cells were washed in RPMI 1640 medium with 10% fetal calf serum (FCS) separated and cell density was adjusted to $2 \times 10^6$/ml. PBMCs were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin in the presence of monensin (ICS-KIT; Hölzel Diagnostica, Laboserv GmbH, Giessen, Germany) to block cytokine secretion [25]. Following a 5-h incubation (37°C, 7% $CO_2$), PBMCs were washed twice with Hank's balance salt solution (HBSS) and fixed (4°C). Fixed PBMCs were washed and treated with a permeabilizing agent for 12 h. Intracellular staining for TNF-$\alpha$, IFN-$\gamma$, IL-2, IL-4, IL-10, and TGF-$\beta$ was performed using specific monoclonal antibodies. Cells were counter stained with an antibody specific for the CD4 cell surface marker (Becton Dickinson, Immunotech, Hölzel, Germany) to identify CD4+ cells. Stained cells were analyzed using a FACScan flow cytometer and cellquest software (Becton Dickinson). Expression of sICAM-1 was measured using an ELISA specific for sICAM (Medgenix, Laboratories, Rungis, France). All lymphocytes were identified by flow cytometry and then cells positive for both CD4 and cytokines of interest were quantified.

### Statistical analyses

Number and volume of $Gd^+$ lesions were analyzed using nonparametric Wilcoxon tests. Differences were considered statistically significant at a $P$-value of 0.05. Overall, seven MRI scans were performed per patient. Results from the two baseline scans were averaged.

## Results

### Patients

Patient demographic and baseline characteristics are presented in Table 1. Of the 10 patients enrolled, six completed the 70-week study. One patient was excluded because of an unplanned pregnancy at week 46; data obtained from this patient were included up to the time of withdrawal. Three other patients discontinued treatment, one each because of side effects and a lack of compliance; the third was lost to follow-up.

**Table 1** Baseline demographic and clinical characteristics of patients ($n = 10$)

| Characteristic | |
|---|---|
| Sex, *n* (%) | |
| Female | 5 (50) |
| Age (years) | |
| Median | 29 |
| Range | 28–38 |
| Relapse rate (preceding 12 months) | |
| Median | 2 |
| Range | 1–3 |
| Years since first event | |
| Median (years) | 4.5 |
| Range | 1–11 |
| EDSS | |
| Median | 2.0 |
| Range | 2.0–4.5 |
| AI | |
| Median | 2 |
| 9-HPT | |
| Median (right) | 22 |
| Median (left) | 21 |
| Number of Gd+ lesions[a] | |
| Mean | 11 |
| Range | 2–39 |

AI, Ambulation Index; EDSS, Expanded Disability Status Scale; 9-HPT, nine-hole peg test; Gd+, gadolinium-enhancing.
[a]Calculated based on $n = 7$.

### Safety and tolerability

The most common adverse events were gastrointestinal symptoms (diarrhea, cramps, nausea) and flushing (Table 2). Mild (6/7) to moderate (1/7) gastrointestinal adverse events were experienced by almost all patients during the initial phase of this study; however, these events decreased continually in all patients after 6 weeks. Administration of antacids was necessary in

**Table 2** Overall adverse events ($n = 7$)

| Adverse event | Number of patients |
|---|---|
| Gastrointestinal | 6 |
| Flushing | 5 |
| Elevated liver enzyme levels | 4 |
| Lymphopenia | 3 |
| Vertigo | 1 |
| Eosinophilia | 1 |
| Headache | 1 |
| Increased perspiration | 1 |

several cases. One patient discontinued because of the gastrointestinal effects of FAE. A transient elevation of liver enzyme levels (up to twofold) occurred in four patients, but these elevations were not sufficient to mandate withdrawal from the study. During therapy, one patient presented at a single visit with an eosinophilia of 15% of all leukocytes, which reverted to a slight elevation (4–6%) within the next weeks. All other side effects were generally mild and transient, needing no further treatment and decreasing within the first 12 weeks of treatment.

### MRI

A significant reduction in the number of Gd+ lesions was already observed following 18 weeks of FAE treatment, with a further reduction after 70 weeks of treatment (Fig. 1). The mean number of Gd+ lesions decreased from 11.28 (range 2–39) at baseline to 0.28 (range 0–1) at the end of the 70-week study ($P < 0.02$). The mean number of lesions at weeks 12, 18, and 22 was 4.3 (range 0–10), 1.5 (range 0–4), and 0.57 (range 0–3), respectively. Similarly, median Gd+ lesion volume was significantly decreased from 244.5 $mm^3$ (range 25–649) at baseline to 26.1 $mm^3$ (range 0–57) after 18 weeks of FAE therapy ($P < 0.018$) (Fig. 2). The lesion volume continued to decrease to 8.6 $mm^3$ (range 0–56) at 22 weeks, and this reduction was maintained to 2.14 $mm^3$ (range 0–9) at 70 weeks ($P < 0.018$).

### Clinical outcomes

The six patients who completed the study demonstrated a stable or slightly improved EDSS score over the course of the study (Table 3). Similarly, there was improvement in functional tests using the 9-HPT at 18 weeks, which was sustained to 70 weeks. The AI also improved over the course of the study. The median AI score improved from 2.0 at baseline to 1.0 at 18 weeks, and this improvement was sustained until 70 weeks; however, these changes did not achieve statistical significance.

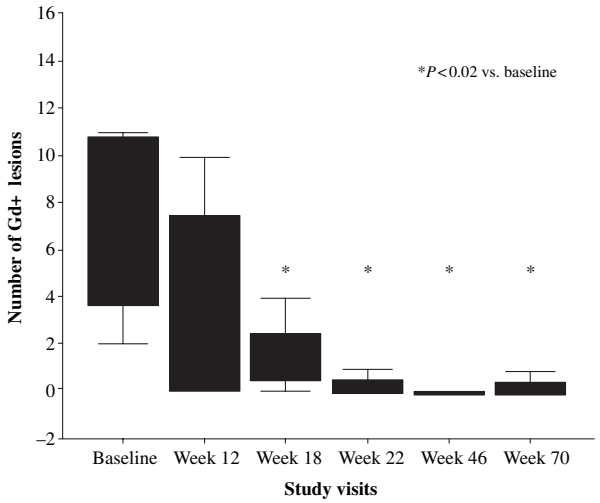


**Figure 1** Mean number of Gd+ lesions from T1-weighted magnetic resonance imaging scans performed at baseline and at weeks 12, 18, 22, 46, and 70.

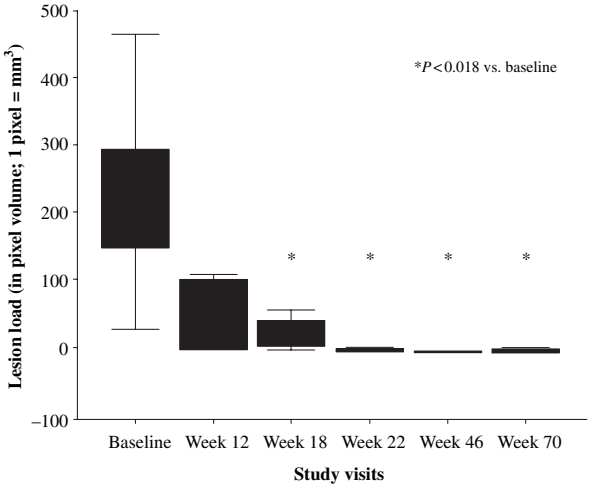


**Figure 2** Total lesion load of Gd+ lesions calculated for all lesions detected at baseline and at weeks 12, 18, 22, 46, and 70. Numbers presented represent total volume of Gd+ lesions detected on T1-weighted magnetic resonance imaging scans as determined following conversion of images into 8-bit grayscale format for quantification. Bars represent median volume of Gd+ lesions; at week 70, only six patients were examined.

**Table 3** Clinical outcomes

| Outcome | Baseline | Week 12 | Week 18 | Week 22 | Week 46 | Week 70[a] |
|---|---|---|---|---|---|---|
| EDSS (median) | 2.0 | 2.0 | 1.5 | 1.5 | 1.5 | 1.5 |
| AI (median) | 2.0 | 2.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 9-HPT (median; in s; right) | 22 | 20 | 20.5 | 17 | 18 | 19 |
| 9-HPT (median; in s; left) | 21 | 20.5 | 20.5 | 18 | 19 | 19 |

AI, Ambulation Index; EDSS, Expanded Disability Status Scale; 9-HPT, nine-hole peg test.
[a]$n = 6$.

Relapse occurred during treatment in two patients at weeks 18 and 46. In both cases, the EDSS score increased by 0.5. Both patients were treated with intravenous corticosteroids (1000 mg methylprednisolone i.v. for 3 days), which resulted in complete remission.

### Cytokine expression and apoptosis

The expression of Th2 cytokine, such as IL-10, in $CD4^+$ lymphocytes, increased during treatment

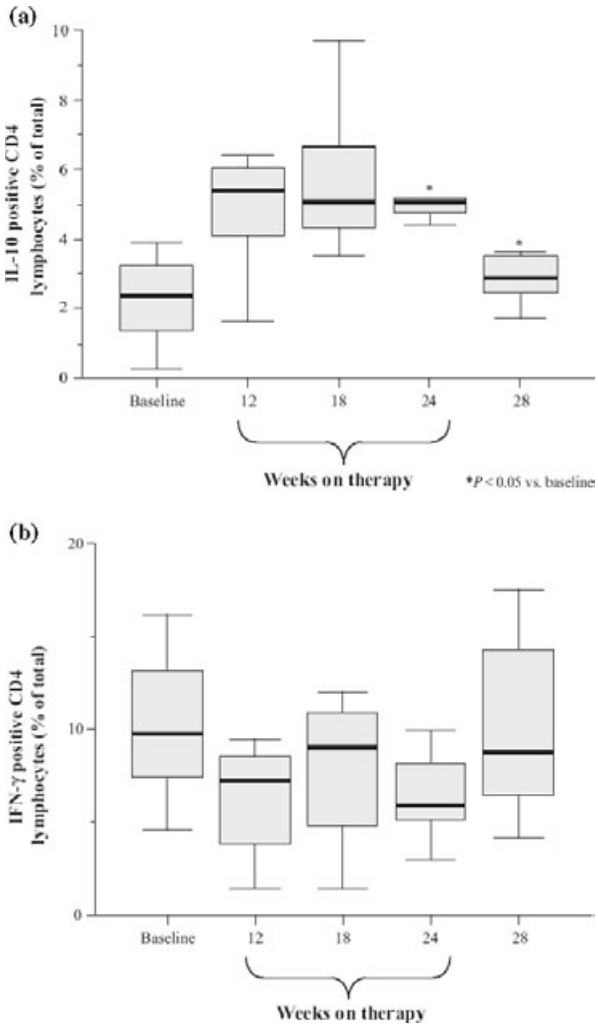


**Figure 3** Expression of Th1- and Th2-like cytokines examined in $CD4^+$ T cells by flow cytometry. (a) Expression levels of interleukin-10 over the course of the study. $*P < 0.05$ vs. baseline. (b) Expression levels of interferon-$\gamma$ over the course of the study. No significant differences from baseline levels were observed at any time point.

(Fig. 3a). Increases in IL-10 levels at weeks 24 and 28 were statistically significant ($P < 0.05$) compared with baseline. In contrast, the expression of Th1-type cytokines such as IFN-$\gamma$ was not significantly affected (Fig. 3b). The rate of apoptosis increased in both the total lymphocyte population and $CD4^+$ lymphocyte population, from 7% at baseline to 11% at week 6 (Fig. 4). In the total lymphocyte population the apoptotic rate was 12% at baseline and increased to 17% at week 6 (not shown). The increases in apoptotic rates were not sustained, and by week 12 the rate of apoptosis had returned to baseline levels in both the total lymphocyte population and $CD4^+$ population.

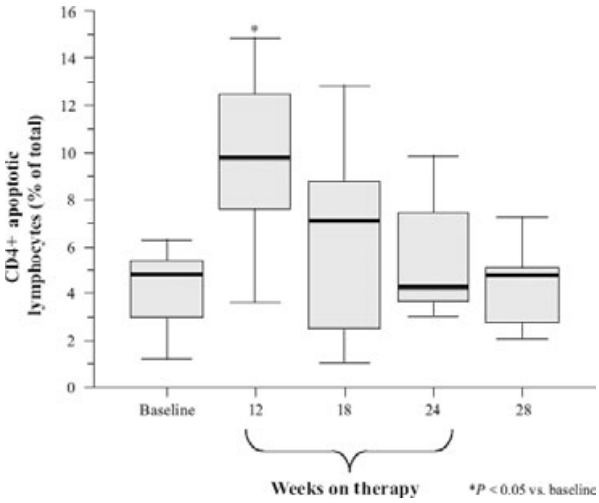


**Figure 4** Rate of apoptosis in $CD4^+$ T-cell population over the course of the study. $*P < 0.05$ vs. baseline.

## Discussion

This report describes the first evaluation of FAE for the treatment of patients with RRMS. The study period included more than 3400 patient-days of observation. Treatment of psoriasis with FAE has been associated with mild to moderate gastrointestinal side effects [19], although in patients with psoriasis these effects are generally mild and subside with time on therapy. In this preliminary study, FAE therapy was well tolerated by patients with MS. Side effects were similar to those experienced by patients treated with FAE for psoriasis and included gastrointestinal symptoms (diarrhea, cramps) and flushing.

A significant decrease from baseline was seen in both the number and volume of Gd+ lesions starting after 18 weeks of treatment. All clinical measures (EDSS, AI, and 9-HPT) either remained stable or showed improvement during the study, which further supports the MRI findings. However, the interpretation of these results is limited by the small number of patients in this study. The fact that FAE treatment reduced brain lesions to a degree that reached statistical significance in a study with a small number of patients is encouraging and suggests that larger trials to determine the efficacy of FAE in patients with MS should be conducted. Additional MRI measurements to detect brain atrophy and new MRI techniques that provide greater sensitivity for the detection of brain lesions [26] may provide insight into the potential effects of FAE on disease activity in patients with RRMS. Given the baseline-controlled nature of this study, the possibility that patients were recruited during a period of high disease activity must be

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