

# Effects of fluoxetine on disease activity in relapsing multiple sclerosis: a double-blind, placebo-controlled, exploratory study

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## ABSTRACT

**Background:** Suppressing the antigen-presenting capacity of glial cells could represent a novel way of reducing inflammatory activity in multiple sclerosis (MS).

**Aims:** To evaluate the effects of fluoxetine on new lesion formation in patients with relapsing MS.

**Methods:** In a double-blind, placebo-controlled exploratory study, 40 non-depressed patients with relapsing remitting or relapsing secondary progressive MS were randomised to oral fluoxetine 20 mg or placebo daily for 24 weeks. New lesion formation was studied by assessing the cumulative number of gadolinium-enhancing lesions on brain MRI performed on weeks 4, 8, 16 and 24.

**Results:** Nineteen patients in both groups completed the study. The mean (SD) cumulative number of new enhancing lesions during the 24 weeks of treatment was 1.84 (2.9) in the fluoxetine group and 5.16 (8.6) in the placebo group ( $p = 0.15$ ). The number of scans showing new enhancing lesions was 25% in the fluoxetine group versus 41% in the placebo group ( $p = 0.04$ ). Restricting the analysis to the past 16 weeks of treatment showed that the cumulative number of new enhancing lesions was 1.21 (2.6) in the fluoxetine group and 3.16 (5.3) in the placebo group ( $p = 0.05$ ). The number of patients without enhancing lesions was 63% in the fluoxetine group versus 26% in the placebo group ( $p = 0.02$ ).

**Conclusions:** This proof-of-concept study shows that fluoxetine tends to reduce the formation of new enhancing lesions in patients with MS. Further studies with this compound are warranted.

**Trial registration:** Current Controlled Trials, ISRCTN65586975

Focal inflammatory demyelinating lesions in multiple sclerosis (MS) are believed to result from autoreactive T-cell-mediated processes.<sup>1,2</sup> The inflammatory cascade and the resultant lesion formation in MS are contingent on the ability of activated anti-myelin T cells to recognise their specific antigen in the context of class II major histocompatibility complex (MHC) molecules expressed on the membrane of antigen-presenting glial cells.<sup>1,2</sup> Medications currently used or under development for the treatment of MS target the peripheral immune system. Inhibition of MHC class II expression on glial cells would represent a novel therapeutic approach to suppress disease activity in MS.

Whether microglia or astrocytes represent the principal CNS antigen-presenting cells in MS remains a controversial issue. Many neuroimmunologists

consider microglia as the primary immunoeffector cells in MS because they constitutively express MHC class II antigens.<sup>3</sup> An alternative hypothesis proposes that astrocytes play an important role as facultative antigen-presenting cells.<sup>4</sup> In contrast to microglia, MHC class II expression on astrocytes under normal conditions is severely restricted by regulatory influences, some of which are mediated by intracellular cAMP signalling pathways.<sup>5,6</sup> A role of astrocytes as facultative antigen-presenting cells in MS is supported by the findings that scattered astrocytes in active MS lesions express MHC class II and B-7 costimulatory molecules.<sup>7,8</sup> Our group has postulated the hypothesis that this may be attributed to reduced cAMP signalling in astrocytes, caused by a loss of  $\beta_2$  adrenergic receptors.<sup>4,9,10</sup>

The objective of this study was to provide proof of concept that enhancing intracellular cAMP signalling pathways in astrocytes in patients with MS reduces inflammatory disease activity. We selected fluoxetine, which is a selective serotonin-reuptake inhibitor (SSRI) that is widely prescribed for depression, bulimia and obsessive-compulsive disorders. Astrocytes, including those in MS lesions, contain serotonin receptors and reuptake sites, and serotonin has been shown to elevate cAMP levels in astrocytes.<sup>11-13</sup> Furthermore, prolonged exposure to SSRIs activates intracellular cAMP signalling in the CNS of animals.<sup>14,15</sup> We conducted a double-blind, placebo-controlled, exploratory study to assess the effects of fluoxetine on inflammatory disease activity in patients with a relapsing form of MS by using serial gadolinium-enhanced brain MRI.

## METHODS

### Subjects

The local medical ethics committee approved the protocol. All patients provided written informed consent. Eligible patients were aged 18–65 years with clinically definitive relapsing remitting or relapsing secondary progressive MS.<sup>16,17</sup> Additional inclusion criteria were an Expanded Disability Status Score (EDSS) of less or equal to 6, and at least one relapse in the preceding year, or two relapses in the preceding 2 years, or one gadolinium-enhancing lesion on the screening MRI of the brain. Exclusion criteria were the use of immunomodulatory, immunosuppressive or antidepressant drugs in the previous 6 months, the use of corticosteroids in the previous 8 weeks, depression defined as a score of 19 or higher on Beck's Depression Inventory II,<sup>18</sup> bipolar disorder, contraindication to

**Table 1** Patient characteristics.

	Fluoxetine (n = 19)	Placebo (n = 19)	p Value
Mean age; years (SD)	41 (10)	38 (9)	0.34
Sex; M/F	9/10	9/10	1.00
Disease course: RR/SP	18/1	16/3	0.60
Mean time from first symptoms; years (SD)	11 (7)	11 (8)	0.97
Median number of exacerbations in the past 2 years (range)	2 (1–3)	2 (0–3)	0.62
Median EDSS (range)	3.0 (0.0–6.0)	3.0 (1.0–5.5)	0.95
Mean MSFC (SD)	0.18 (0.6)	0.10 (0.6)	0.70
Number of new enhancing lesions at baseline			0.56
Mean (SD)	0.63 (1.3)	0.58 (0.8)	
Median (range)	0 (0–5)	0 (0–3)	
Scans showing enhancing lesions baseline	7 (37%)	8 (42%)	0.74
T2 lesion load (mm <sup>3</sup> )			0.54
Mean (SD)	4761 (6414)	5527 (6891)	
Median (range)	1894 (670–20829)	2946 (80–28496)	

EDSS, Expanded Disability Status Scale; MSFC, Multiple Sclerosis Functional Composite; RR, relapsing remitting; SP, secondary progressive.

MRI, other neurological or systemic disorder that would interfere with the assessments, and pregnancy or unwillingness to use acceptable birth control.

**Study design**

In this single-centre, double-blind, placebo-controlled study, 40 patients were randomised to receive a single tablet of fluoxetine 20 mg or identical placebo daily in the morning for 24 weeks. After a screening visit and brain MRI 4 weeks prior to start of the study medication, patients visited the clinic for brain MRI and clinical evaluation at weeks 0, 4, 8, 16 and 24. Disability status was assessed at baseline and week 24. The hospital pharmacy produced the study medication and performed the randomisations. The code was revealed to the researchers once the follow-up of all patients and the MRI analyses were completed. One physician was responsible for all clinical

assessments. Relapses could be treated with high-dose corticosteroids for 5 consecutive days.

**MRI protocol and processing**

All scans were performed on a 3.0 Tesla scanner (Philips). Brain transaxial Dual TSE (repetition time, 3000 ms; echo times, 26.7 and 120 ms), FLAIR (repetition time, 11,000 ms; echo time, 100 ms) and T1-weighted (repetition time, 700 ms; echo time, 8.4 ms) images before and after intravenous administration of gadolinium (0.1 mmol/kg) with 51 contiguous slices of 3 mm thickness were obtained at each visit.

The scans were blindly analysed in the Department of Radiology of the Leiden University Medical Center. T1 enhancing lesions were determined according to published guidelines.<sup>19</sup> T2 lesion volume was segmented with a semi-automated home-developed software program called SNIPER.<sup>20</sup>

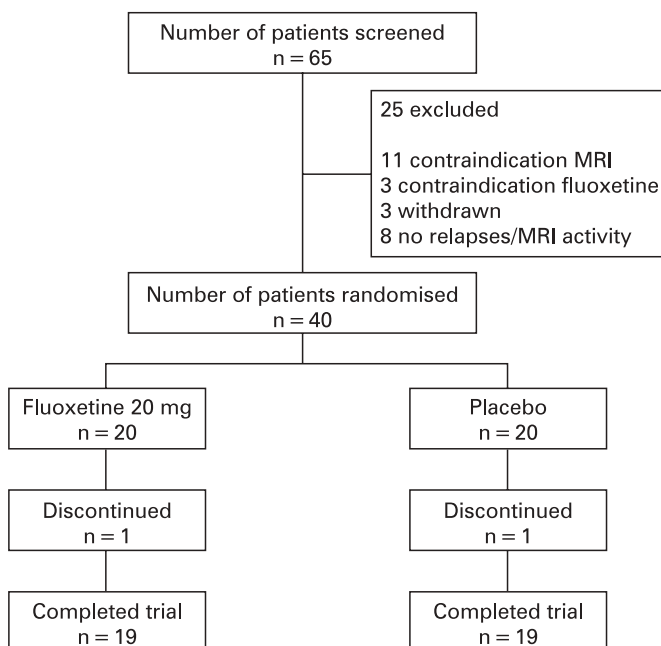
**Outcome measures**

The primary outcome measure was the cumulative number of new gadolinium-enhancing lesions during the treatment phase. Other MRI outcome measures included the number of scans showing new enhancing lesions, the number of scans showing enhancing lesions, the number of patients with no enhancing lesions, the change in T2 lesion volume and cumulative new T1 gadolinium enhancing lesion volume.

Secondary clinical endpoints were number of relapses, number of patients with relapses, and changes in EDSS and Multiple Sclerosis Functional Composite (MSFC). The MSFC is a multidimensional test consisting of a task for leg function (timed 25-foot walk), arm function (9-hole peg test) and cognition (paced auditory serial addition test). Its score represents the mean of the z-scores of the three tests, which are calculated in comparison to a pooled reference population.<sup>21</sup> Lower scores indicate more disability. Exploratory analyses of MRI data in the first 8 weeks and past 16 weeks were performed separately to assess possible time-dependent effects of fluoxetine treatment.

**Statistical analysis**

Because this was an exploratory study and because it is difficult to define a relevant effect of fluoxetine, we did not perform a power calculation. The aim of our study was to collect information about an effect size, and if any to use this effect size in the design of future studies.



**Figure 1** Patient flowchart.

**Table 2** MRI outcomes over the 24-week study period.

	Fluoxetine (n = 19)	Placebo (n = 19)	p Value
Cumulative number of new enhancing lesions			0.15
Mean (SD)	1.84 (2.9)	5.16 (8.6)	
Median (range)	1 (0–12)	2 (0–35)	
Cumulative volume of new enhancing lesions (mm <sup>3</sup> )			0.16
Mean (SD)	124 (278)	398 (745)	
Median (range)	22 (0–1191)	77 (0–3063)	
Number of patients with no new enhancing lesions	6 (32%)	4 (21%)	0.71
Scans showing new enhancing lesions	19 (25%)	31 (41%)	0.04
Scans showing enhancing lesions	22 (29%)	33 (43%)	0.06
Change in T2 lesion load (mm <sup>3</sup> )			0.10
Mean (SD)	444 (958)	531 (1004)	
Median (range)	128 (–506–2930)	475 (–1907–2391)	

All data were tested for normality. Baseline and between-treatment comparisons of the number and volume of T1 enhancing lesions, T2 lesion load and EDSS were evaluated with the Wilcoxon–Mann–Whitney rank-sum test. For baseline and between-treatment comparisons of the MSFC, the independent samples *t*-test was used. The  $\chi^2$  test and Fisher's exact test were used to compare differences in categorical variables. Analyses were performed with the Statistical Package for the Social Sciences (SPSS 14.0 for Windows). All reported *p* values are two-tailed. Significance was taken at 0.05.

## RESULTS

### Patients

Between April 2004 and August 2006, 65 patients were screened, after which 40 were found eligible for inclusion. Figure 1 shows the flow of the patients. In the first week after randomisation, one patient in the fluoxetine group and one patient in the placebo group withdrew because of nausea. All other patients completed the study and were used in the analyses. There were no significant differences between the two groups in baseline MRI, clinical or demographic characteristics (table 1).

### Efficacy

Table 2 shows MRI outcomes over the 24-week study period.

The mean cumulative number of new enhancing lesions tended to be lower in the fluoxetine group than in the placebo group, but this was not significant ( $p = 0.15$ ). However, compared with the placebo group, there were significantly less scans with new enhancing lesions in the fluoxetine group ( $p = 0.04$ ). The fluoxetine group also showed a trend towards a reduction in the cumulative volume of new enhancing lesions, the number of scans with enhancing lesions and increase of the T2 lesion load.

Figure 2 shows the time-dependent changes in mean cumulative number of new enhancing lesions from the start of treatment. Exploratory analysis revealed no differences in MRI outcome measures between the two groups up to the first 8 weeks of treatment. Analysis of disease activity during the past 16 weeks (table 3) showed a nearly significant reduction in the cumulative number ( $p = 0.05$ ) and volume ( $p = 0.06$ ) of new enhancing lesions, and a lower number of scans with enhancing ( $p = 0.03$ ) or new enhancing lesions ( $p = 0.03$ ) in the fluoxetine group compared with the placebo group. The fluoxetine group contained a higher number of patients with no new enhancing lesions ( $p = 0.02$ ).

Number of relapses, and changes in EDSS and MSFC, were comparable between the two groups (table 4).

### Safety and tolerability

In general, fluoxetine was well tolerated. Patients using fluoxetine suffered more often from nausea and drowsiness (table 5). Most adverse events were at the start of the study medication and diminished after a few days to weeks.

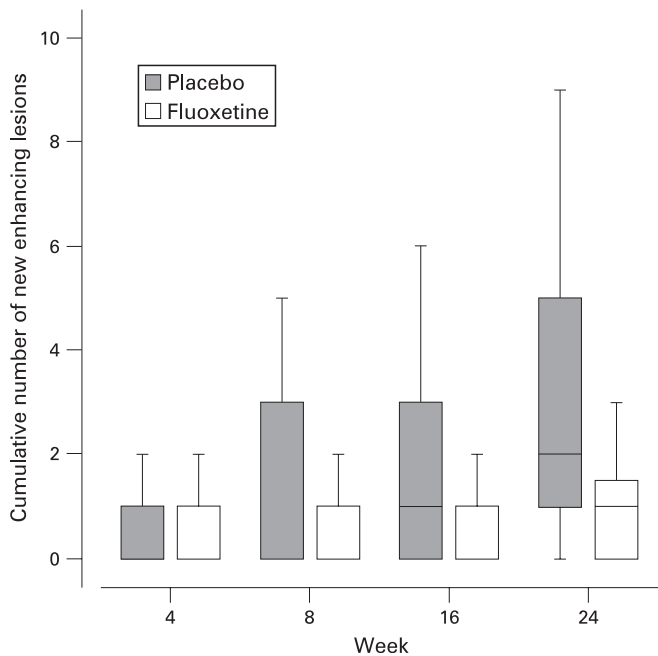
## DISCUSSION

In this study, patients with the relapsing form of MS treated with fluoxetine showed a trend towards a reduction in the number of new enhancing lesions over time. This effect became apparent after 8 weeks of treatment, suggesting that it takes several weeks before fluoxetine becomes effective. Fluoxetine plasma concentrations gradually build up and achieve steady-state conditions only after several weeks,<sup>22</sup> and it is well known that patients with depression need treatment for a number of weeks before effects become clinically evident.

Conclusions from our results must be made with caution because of the small sample size and exploratory design.

**Table 3** MRI outcomes during the past 16 weeks.

	Fluoxetine (n = 19)	Placebo (n = 19)	p Value
Cumulative number of new enhancing lesions			0.05
Mean (SD)	1.21 (2.6)	3.16 (5.3)	
Median (range)	0 (0–11)	1 (0–22)	
Cumulative volume of new enhancing lesions (mm <sup>3</sup> )			0.06
Mean (SD)	90 (231)	227 (485)	
Median (range)	0 (0–961)	35 (0–2095)	
Number of patients with no new enhancing lesions	12 (63%)	5 (26%)	0.02
Scans showing new enhancing lesions	9 (24%)	18 (47%)	0.03
Scans showing enhancing lesions	9 (24%)	18 (47%)	0.03



**Figure 2** Boxplots of the cumulative number of new enhancing lesions per treatment group over time.

Retrospective power calculation shows that we were only able to detect treatment effects above 80% with a statistical power of 80%.<sup>23</sup> The treatment with fluoxetine was shown to be safe and well tolerated.

The results lend support to the hypothesis that elevating cAMP signalling in astrocytes may reduce inflammatory disease activity in patients with MS.

An increase in cAMP signalling in astrocytes downregulates the class II transactivator protein,<sup>24</sup> thereby suppressing the induction by proinflammatory cytokines of MHC class II molecules.<sup>25</sup> This mechanism is thought to prevent the deviation of astrocytes to function as facultative immunocompetent CNS antigen-presenting cells that can mediate

inflammatory events. Possible influences of fluoxetine on microglia or cells of the peripheral immune system cannot be dismissed, but are unlikely. In vitro studies showed that elevation of intracellular cAMP levels suppresses interferon- $\gamma$  induction of MHC class II in astrocytes, but not in microglial cells.<sup>6</sup> In vitro experiments on human lymphocyte suspensions showed that fluoxetine decreases lymphocyte proliferation and suppresses interferon- $\gamma$  production.<sup>26</sup> However, these effects were only found with fluoxetine concentrations of 10–50  $\mu$ M, which are far in excess of therapeutic plasma concentrations. Serum levels of fluoxetine with daily doses ranging from 20 to 40 mg vary between 0.26 and 0.65  $\mu$ M,<sup>27</sup> making it unlikely that immunomodulatory effects of fluoxetine on T cells were involved in our study.

An interesting aspect of considering fluoxetine as a candidate drug for the treatment of MS are its additional mechanisms of action that might be relevant for reducing axonal loss, leading to progressive disability. These include the production by astrocytes of neurotrophic factors, such as brain-derived neurotrophic factor,<sup>28</sup> stimulation of astrocyte glycogenolysis<sup>29</sup> and blockage of sodium channels.<sup>30</sup> Sodium channel activation,<sup>31</sup> and reduced axonal energy metabolism caused by impaired glycogenolysis in astrocytes,<sup>10</sup> have been hypothesised to play a role in the axonal degeneration in MS. In patients with MS, fluoxetine improved the cerebral white matter N-acetylaspartate/creatine ratio, which can be regarded as a measure of axonal energy metabolism.<sup>32</sup>

The results of our exploratory trial are sufficiently encouraging to justify further studies with fluoxetine in patients with MS. Higher doses of fluoxetine and combination treatment with immunomodulatory drugs should be considered.

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**Competing interests:** None.

**Table 4** Clinical outcomes.

	Fluoxetine (n = 19)	Placebo (n = 19)
Number of exacerbations	4	6
Number of patients with exacerbations	4 (21%)	5 (26%)
Median change in EDSS (range)	0.0 (–2.0–1.5)	0.0 (–1.5–1.0)
Mean change in MSFC (SD)	0.075 (0.38)	0.049 (0.14)

EDSS, Expanded Disability Status Scale; MSFC, Multiple Sclerosis Functional Composite.

**Table 5** Adverse events.

	Fluoxetine (n = 20) (%)	Placebo (n = 20) (%)	p Value
Nausea	13 (65)	6 (30)	0.03
Headache	4 (20)	6 (30)	0.72
Dizziness	5 (25)	7 (35)	0.49
Drowsiness	11 (55)	6 (30)	0.11
Insomnia	2 (10)	2 (10)	1.00
Transpiration	2(10)	1 (5)	1.00
Palpitations	2 (10)	–	0.49
Loss of appetite	2 (10)	–	0.49

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