Fluoxetine modulates Th17-immune response via 5-HT2B-receptors in multiple sclerosis.

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Introduction

In multiple sclerosis (MS), psychological stress may enhance the production of pro-inflammatory cytokines and promote exacerbations of the disease. Serotonin (5-hydroxytryptamine (5-HT) is one of the key neurotransmitters in CNS that is involved in developing psychological and cognitive impairments Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), which also has an immunomodulatory effect.[1]

Objective

This study aimed to clarify the effects of SSRI fluoxetine on Th17-immune response in MS.

Materials and methods

Patients

Thirty patients with a documented diagnosis of MS according to the McDonald criteria (modification 2010) were examined.[2] All patients had a relapsing–remitting form of the disease. Their main demographic and clinical characteristics are shown in **Table 1**. All patients were subjected to a standard neurological examination with assessment of the EDSS score.[3] All patients were non-smoking and had no mental disorders, according to the Beck Depression Inventory and cognitive impairments, according to Montreal Cognitive Assessment. All patients were examined during clinical remission. All patients had been treated with glatiramer acetate for more than one year. At the time of blood sampling, all the patients studied had not been treated with corticosteroid or SSRIs therapy for more than six months. The control group consisted of twenty healthy donors matched with patients by sex and age (**Table 1**). All patients signed the written informed consent to participate in this study. The study was approved by the ethics committee of the Russian National Research Medical University (protocol №192).

Flow cytometry

The percentage of circulating Th17-cells was determined by flow cytometry. Th17-cells were identified as $CD4^+CD26^+CD161^+$ events and quantified as percentage of these events in relation to all $CD4^+$-T-cells.[4,5]

CD4+ T-cells cultures and stimulation

To determine functional activity of Th17- and Th1-cells, $CD4^+$ T-cells were isolated from PBMCs by magnetic cell sorting (Milteny Biotec, Germany). Then $CD4^+$ T-cells at a concentration of 8×10^4 per 200 µl per well were plated in 96-well U-bottomed culture plates in duplicate and stimulated with anti-CD3/anti-CD28-microbeads (Life Technologies, Norway) for 72 hours whereafter culture supernatants were collected and stored at –70° C.

To assess the effect of fluoxetine on Th17-cells function, $CD4^+$ T-cells were cultured in the presence of fluoxetine (Tocris, Switzerland) at a concentration of 10^{-6} M whereafter anti-CD3/anti-CD28 microbeads were added to the cultures. To study the involvement of $5\text{-}HT_{2B}$-receptors in fluoxetine-mediated modulation of cytokine production, some samples of $CD4^+$ T-cells were pre-incubated with antagonist (RS 127445) or agonist (BW 7233C86) of $5\text{-}HT_{2B}$-receptors (Tocris, Switzerland) (both at 10^{-6} M) whereafter fluoxetine and anti-CD3/anti-CD28 microbeads were added to the cultures. In addition, in some experiments, $CD4^+$ T-cells were pre-incubated in the presence of antagonist or agonist of $5\text{-}HT_{2B}$-receptors and activated by anti-CD3/anti-CD28 microbeads.

5-HT and 5-hydroxyindolacetic acid evaluation. The level of 5-HT and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in blood plasma and culture supernatants were determined by high-performance liquid chromatography (HPLC). Data are expressed as pmol/ml.

Cytokine evaluation

Levels of IL-17, IFN-γ, and GM-CSF in the supernatants were determined by ELISA (Invitrogen, USA). In all cases of ELISAs, the instructions of the kit manufacturers were followed. Data are expressed as pg/ml or as the percentage of cytokine production by stimulated cells in the absence of fluoxetine and antagonist or agonist of 5-HT2B-receptors.

Statistical analysis

The statistical analysis of the results was performed using Prizm 6 software.

The nonparametric Wilcoxon test was used to compare two groups. Differences were considered statistically significant if $p<0.05$.

Results

The percentages of $CD4^+$ T-cells, Th17-cells as well as IL-17, IFN-γ, and GM-CSF production by activated $CD4^+$ T-cells in MS patients and healthy subjects were comparable **(Table 2, Table 3)**. The concentrations of 5-HT and 5-HIAA were also comparable between the groups (**Table 2**).

Fluoxetine suppressed IL-17, IFN-γ, and GM-CSF production by $CD4^+$ T-cells in both groups **(Fig. 1)** without affecting cell viability and proliferative responses (data not shown). The blockade of $5\text{-}HT_{2B}$-receptors with specific antagonist reduced the inhibitory effect of fluoxetine on IL-17, IFN-γ, and GM-CSF production in MS patients (**Fig 2A**, **Fig 2B** and **Fig 2C**) and GM-CSF in healthy subjects (**Fig 2F**), while activation of $5\text{-}HT_{2B}$-receptors by specific agonist further reduced IL-17 production in both groups (**Fig 2A** and **Fig 2D**) and IFN-γ production in healthy subjects (**Fig 2E**).

Conclusions

These data suggest an anti-inflammatory role of fluoxetine in MS which could be mediated by the $5\text{-}HT_{2B}$-receptors activation.

References

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Disclosure

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Table 1. Clinical and demographic characteristics of MS patients and healthy subjects. Data are medians (25^{th}; 75^{th} percentiles)

Factor	MS patients, n=30	Healthy subjects, n=20
Age, years	29 (24; 32)	30.0 (27.8; 34.5)
Men/women (% women)	10/20 (66.7)	8/12 (60)
Duration of MS, years	2 (2; 5)	NA*
EDSS score	1.5 (1.5; 2)	NA*

* NA – not applicable

Table 2. Distribution of subpopulations of blood CD4+ and Th17-cells (flow cytometry) and concentration of 5-HT and 5-HIAA in blood plasma (HPLC) in MS patients and in healthy subjects.

Factor	MS patients, n=30	Healthy subjects, n=20
$CD4^+$ T-cells, % relative to lymphocytes	39.4 (36.1; 45.9)	39.0 (36.0; 48.0)
$CD4^+CD26^+CD161^+$-Th17-cells, % relative to $CD4^+$ T-cells	15.0 (12.4; 18.0)	18.2 (15.6; 23.0)
5-HT, pmole/ml	609.2 (387.3; 775.4)	669.4 (468.9; 855.4)
5-HIAA, pmole/ml	13.4 (10.9; 18)	7.9 (3.6; 11.5)

Table 3. The secretion of cytokines by $CD4^+$ T-cells in MS patients and in healthy subjects.

Cytokine	Stimulation	MS patients, n=30	Healthy subjects, n=20
IL-17, pg/ml	None	0 (0; 4)	4 (0; 9)
	Anti-CD3 / anti-CD28	732 (321; 1201)	696 (105; 984)
IFN-γ, pg/ml	None	7 (0; 24)	6 (0; 34)
	Anti-CD3 / anti-CD28	4934 (2776; 7377)	4781 (2964; 5519)
GM-CSF, pg/ml	None	0 (0; 6)	0 (0; 9)
	Anti-CD3 / anti-CD28	440 (202; 751)	425 (162; 846)

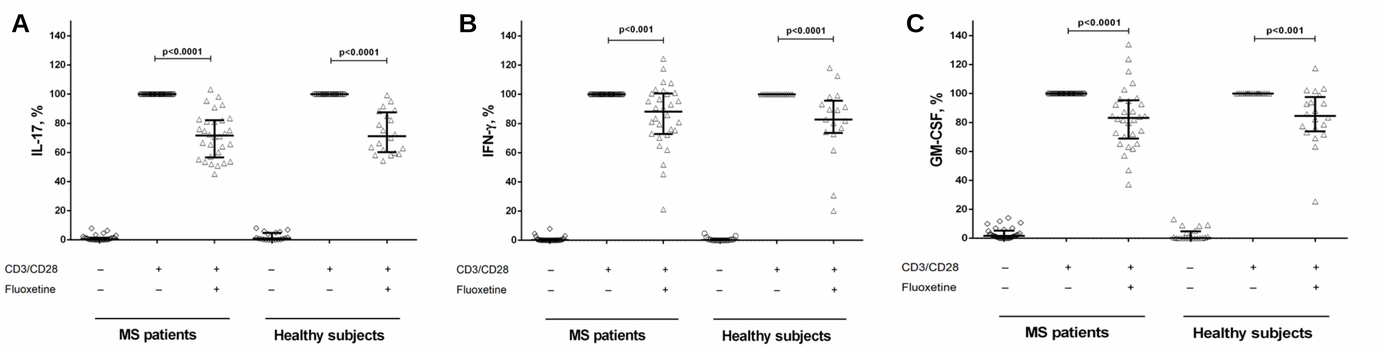

Figure 1. The influence of fluoxetine on IL-17 (A), IFN-γ (B), and GM-CSF (C) production by stimulated $CD4^+$ T-cells in MS patients and healthy subjects. Horizontal lines at the graphs correspond to the median and whiskers indicate to 25th and 75th percentiles.

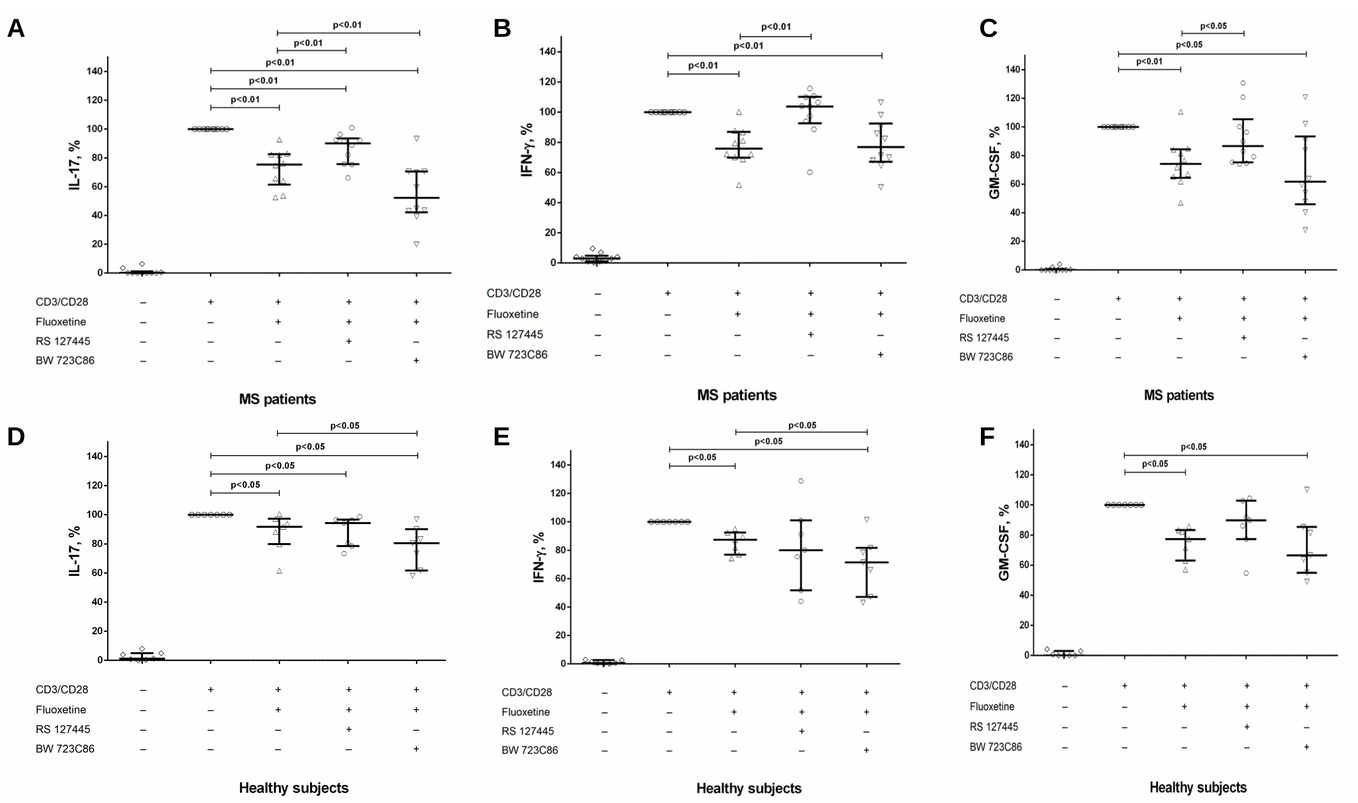

Figure 2. The role of $5\text{-}HT_{2B}$-receptors in fluoxetine-mediated suppression of IL-17, IFN-γ, and GM-CSF production by stimulated $CD4^+$ T-cells in MS patients (A, B, C) and healthy subjects (D, E, F).

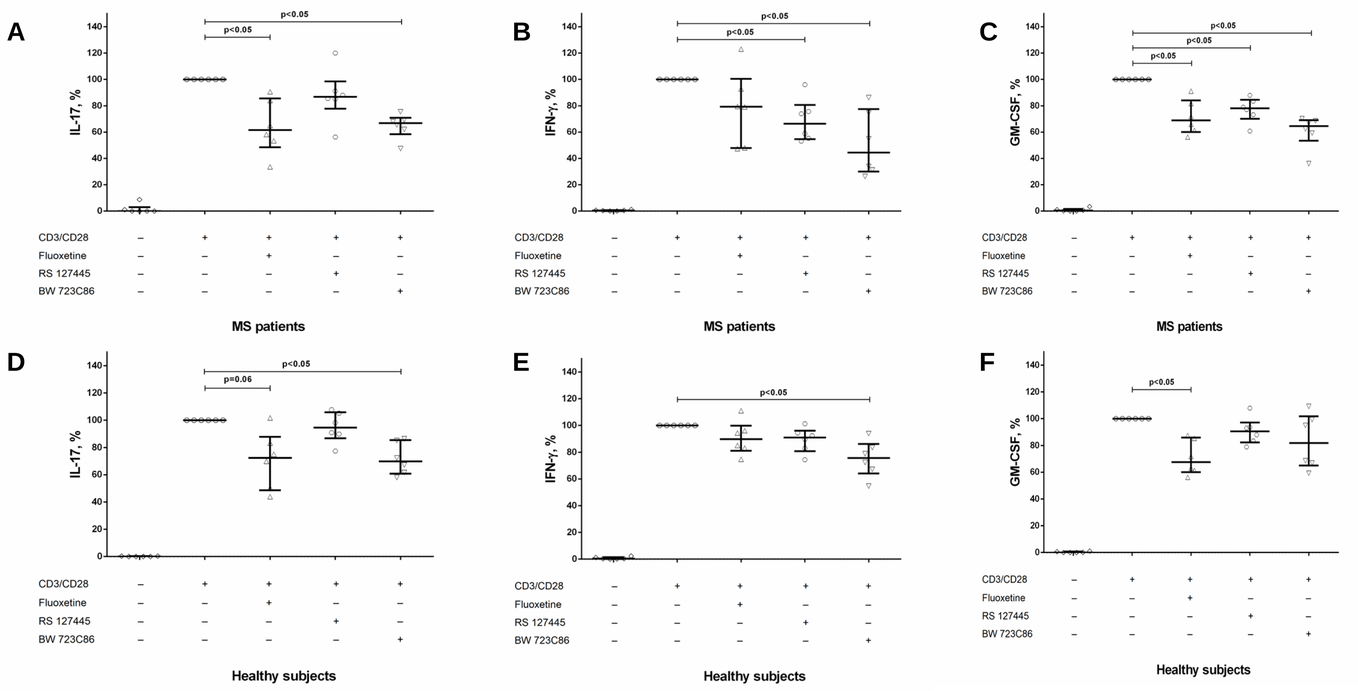

Figure 3. The influence of $5\text{-}HT_{2B}$-receptors agonist on IL-17, IFN-γ, and GM-CSF production by stimulated $CD4^+$ T-cells in MS patients (A, B, C) and healthy subjects (D, E, F).