Sativex® effects on promoter methylation and on CNR1/CNR2 expression in peripheral blood mononuclear cells of progressive multiple sclerosis patients

Losavio FA[1], Santoro M[2] , Mirabella M[1], De Fino C[1], Bianco A[1], Lucchini M[1], Sabino A[2], Nociti V[1,2]

- [1] Fondazione Policlinico Universitario "A. Gemelli", Catholic University of Sacred Heart, L.go F. Vito 1, 00168, Rome, Italy
- [2] Fondazione Don C. Gnocchi, Via A. Capecelatro 66, 20148 , Milan, Italy

OBJECTIVES

Sativex® is a 1:1 mix of 9-delta-tetrahydrocannabinol (THC) and cannabidiol (CBD) extracted from cloned Cannabis sativa chemovars. It is available as an oromucosal spray for the treatment of spasticity in MS patients. The main target of THC and CBD are the cannabinoid receptors named CB1 (encoded by the CNR1 gene) and CB2 (encoded by the CNR2 gene). The CB1 receptors are dominantly expressed in the central nervous system (CNS), whereas the CB2 ones are primarily expressed in immune and microglial cells and are involved in cytokine secretion, immune cell trafficking and cell survival of peripheral blood mononuclear cells (PBMCs).
The aim of our study was to analyze the mRNA expression and the promoter methylation of CNR1 and CNR2 in PBMC of secondary progressive multiple sclerosis (SP-MS) patients before and after Sativex® therapy.

METHODS

Thirty SP-MS patients were included in this study: 10 men, 20 women, mean age 54.2±11.7 years, mean disease duration of 15.4±8.5 years and Expanded Disability Status Scale (EDSS) score of 6.4±1. Peripheral blood leukocytes were collected before the beginning of the treatment with Sativex® (T0) and after one (T1) and three months (T3) from the start. Considering that the expression levels of cannabinoid receptors could be regulated by IFN-β-1b [1], we divided the cohort of patients into three subgroups based on the IFN-β-1b treatment: MS-1 (7 MSS-SP patients treated with IFN-β-1b during the therapy with Sativex®), MS-2 (12 MSS-SP patients that suspended IFN-β-1b before the beginning of the treatment) and MS-3 (11 MSS-SP patients never treated with IFN-β-1b).

RESULTS

We performed MS-HRM on the three groups of SP-MS patients (MS-1, MS-2 and MS-3) at different times of Sativex® treatment (T0, T1 and T3) to determine the methylation levels of CNR1 and CNR2 in the promoter region. We found out that in all groups of SP-MS patients at T0, T1 and T3 the CNR1 is invariably unmethylated (0–10% of methylation). We obtained the same results performing MS-HRM analysis on the promoter region of CNR2.
We performed qRT-PCR on leukocytes to analyze the RNA levels of CNR1 and CNR2 in the three groups of SP-MS patients (MS-1, MS-2 and MS-3) at T0, T1 and T3. We found out that all three groups of SP-MS patients showed at T0 a level of CNR2 mRNA expression higher than CNR1 with a 3:1 ratio.
We performed ANOVA for repeated measures for each SP-MS patients group and we found a significant CNR2 expression level reduction only in MS-1 group comparing T1 vs. T0 ($p = 0.02$) and T3 vs. T0 ($p = 0.01$) (Figure 1). However the expression changes of this gene did not correlate with the CpG methylation levels of the promoter.

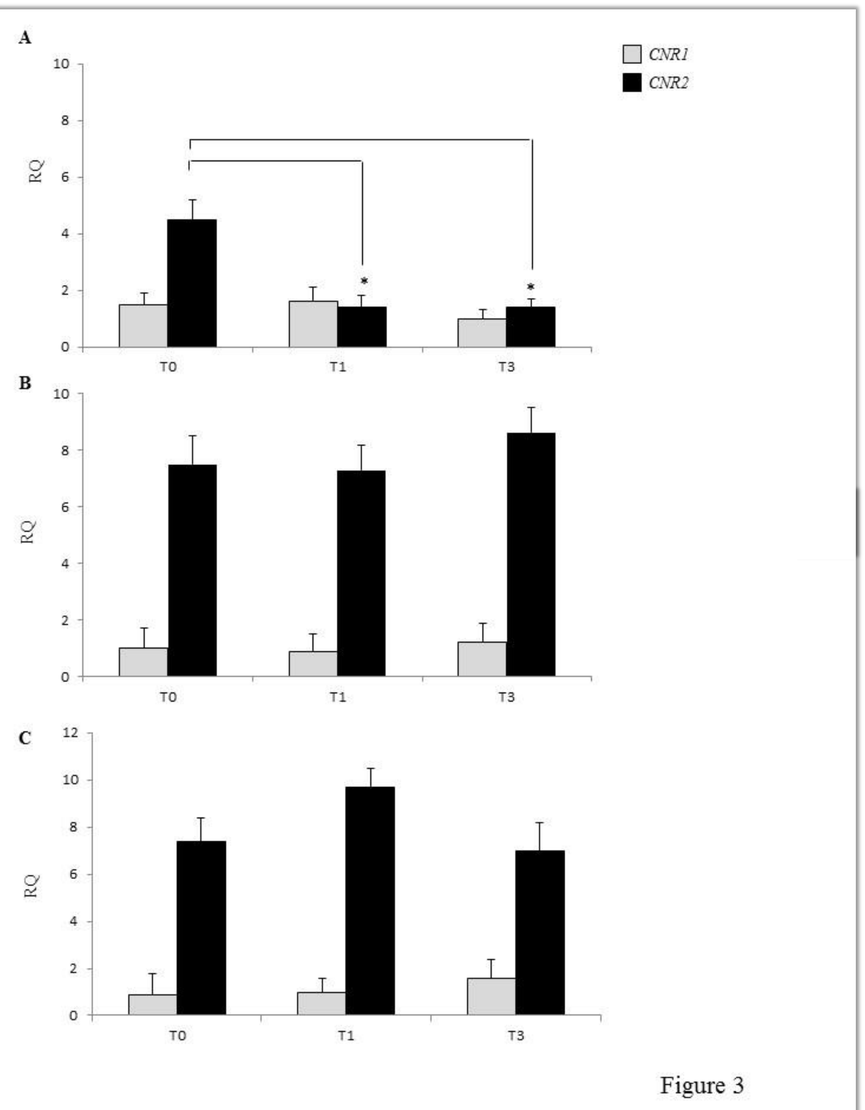

Figure 3

Figure 1: qRT-PCR analysis of CNR1 and CNR2 to determine the relative mRNA transcript levels of CNR1 and CNR2 in the three groups of SP-MS patients. A) MS-1, B) MS-2, C) MS-3. ±standard error (SE). *: $p < 0.05$.

CONCLUSIONS

This study shows that Sativex® therapy does not influence the methylation level of the CNR1 and CNR2 gene promoter regions in SP-MS patients but the patients treated at the same time with IFN-β-1b and Sativex © show a specific decrease of the CNR2 mRNA expression during a 3 months follow-up period while CNR1 mRNA expression level does not significantly change during this time. The CNR2 mRNA expression level reduction does not correlate with the promoter methylation status.
Considering the CB2 receptor involvement in the immune modulation of PBMCs cells, it is possible that Sativex®, together with IFN-β-1b, could act as an anti-inflammatory agent in lymphoid tissue.

REFERENCE

[1] A.J. Sánchez López, L. Román-Vega, E. Ramil Tojeiro, A. Giuffrida, A. García-Merino. Regulation of cannabinoid receptor gene expression and endocannabinoid levels in lymphocyte subsets by interferon-β: a longitudinal study in multiple sclerosis patients, Clin. Exp. Immunol. 179 (2015) 119–127.