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Elevated melatonin levels in natalizumab-treated female patients with relapsing-remitting multiple sclerosis: Relationship to oxidative stress

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ABSTRACT

Natalizumab is currently the most successful clinical treatment for multiple sclerosis. The use of this drug is associated with the reduction in the number of relapses and a slowing in disease progression, as well as an improvement in signs and symptoms displayed by the patients. To evaluate the effect of natalizumab on melatonin and its relationship with peripheral oxidative damage, we studied the serum melatonin levels in 18 patients with relapsing-remitting multiple sclerosis. Natalizumab caused significant increases in serum melatonin concentrations. This change was associated with a rise in increase of antioxidants and a reduction in oxidative stress biomarkers. In conclusion, these data may explain, at least in part, some of the beneficial effects exhibited by disease antibody such as its antioxidant capacity.

1. Introduction

The most common cause of non-traumatic neurological disorder in young adults is multiple sclerosis. Its pathogenesis involves inflammation, oxidative stress, demyelinization and neuronal loss (Compston and Coles, 2002, 2008; Miller, 2012). There are four types of MS: (i) progressive relapsing, (ii) primary progressive, (iii) relapsing-remitting, and (iv) secondary progressive (Compston and Coles, 2002, 2008).

Oxidative stress seems to play an important role in this neurodegenerative condition. Thus, reactive oxygen species have a relevant function in multiple sclerosis pathogenesis (Melamud et al., 2012; Miller et al., 2012; Tasset et al., 2012a, 2012b). Our recent studies found that multiple sclerosis patients have elevated levels of oxidative stress biomarkers, together with a global antioxidant deficiency. This supports the hypothesis that an imbalance between reactive oxygen species and antioxidant system precedes the inflammatory response, at least, in terms of a relapse (Tasset et al., 2012a, 2012b).

Environmental factors have important role in multiple sclerosis pathogenesis (Ghorbani et al., 2013; Hedstrom et al., 2011). Studies have shown a reduction in blood melatonin (N-acetyl-5-methoxytryptamine) levels and dysregulation of its synthesis, secretion and circadian rhythm associated with fatigue, depression and sleep disorder in multiple sclerosis patients (Akpinar et al., 2008; Compston and Coles, 2002; Ghorbani et al., 2013; Melamud et al., 2012; Sandyk and Awerbuch, 1993). Melatonin produced by pineal gland and elsewhere is a potent free radical scavenger and antioxidant (Fischer et al., 2013; Galano et al., 2011, 2013; Miller et al., 2012) and, as such, it may be protective against the neural damage associated with multiple sclerosis.

Natarajan et al. (2012) reported that the melatonin pathway genes are involved in progression of multiple sclerosis. In addition, a recent study found that its administration reduced oxidative status associated to multiple sclerosis. This was accompanied by a reported improvement in functional characteristics of patients, evaluated by the Expanded Disability Status Scale, though the difference could not be statistically verified (Natarajan et al., 2012). These data are consistent with the idea that this, melatonin, may play an important role in the development and progression of multiple sclerosis.

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To date, there is no cure for multiple sclerosis, so treatments are aimed at preventing relapses and mitigating the signs and symptoms associated with this disease. A variety of drugs are used in therapy; natalizumab (Tysabri®) is reported to be the most effective (Cadavid et al., 2013; Phillips et al., 2011; Stephenson et al., 2012; Wickstrom et al., 2013), whereas the most common is interferon-beta.

With this background, we addressed the idea that the changes produced after treatment with natalizumab may be due, in part, to the stabilization of melatonin levels and its antioxidant effects.

2. Materials and methods

Eighteen patients (5 men and 13 women) with relapsing-remitting multiple sclerosis were recruited for the study from the Department of Neurology at Queen Sofia University Hospital in Cordoba. The revised McDonald (Polman et al., 2005) criteria were used and the patients were treated with 300 mg natalizumab (anti-VLA-4; Tysabri, Biogen Idec, Cambridge, MA, USA) administered via intravenous infusion every 4 weeks (28 days) in concordance with current Spanish guidelines during 56 weeks (MS-56) (Fig. 1). The infusions were given between 16:00 and 18:00 hours. Clinical examination was performed using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

Peripheral blood samples were taken immediately prior to the first infusion (baseline) and before the fourteenth infusion. Blood was collected between 15:30 and 17:30 hours in chilled BD Vacutainer® tubes (Becton-Dickinson and Company, BD, Franklin Lakes, NJ, USA) without anticoagulant (for serum) or with anticoagulant, EDTA-K2 (for plasma and erythrocytes). Thereafter serum or plasma samples were immediately separated by centrifugation at 1500 g at 4 °C for 15 min and the fraction was frozen in aliquots and stored at −85 °C.

2.1. Biochemical parameters

Serum samples were tested for melatonin using enzyme immunoassay (Melatonin ELISA) kits according to the manufacturer's instructions (GenWay Biotech Inc., San Diego, CA, USA).

The quantity of the oxidative DNA adduct 8-hydroxy-2'deoxyguanosine (8-OHdG) was evaluated using the assay kit (8-OHdG Check-437-0122) purchased from JaICA (Japan Institute for the Control of Aging, Fukuroi city Shizuoka, Japan). Reduced glutathione (GSH) levels were evaluated using the Bioxytech GSH-400 kit (Oxis International, Portland, OR, USA). The GSH concentration is based on a reaction which leads to the formation of a chromophore with absorbance at 400 nm. The total antioxidant capacity (PAO, KPA-050) was evaluated using a kit purchased from JaICA (Japan Institute for the Control of Aging, Fukuroi City Shizuoka, Japan); this assay is based on the reduction of Cu^{2+} to Cu^{+} by the combined action of all of the antioxidants present in the sample. Thus, the chromogenic reagent forms a complex with Cu^{+} which has an absorbance at 490 nm.

For quantitative detection of the soluble vascular cell adhesion molecule-1 (sVCAM-1) an assay kit (Milliplex® MAP Kit, Human CVVD Panel 1 96-Well Plate Assay, Cat. # HCVD1-67AK) purchased from Millipore™ (Millipore Corporation, Concord Road, Billerica, MA, USA) was used.

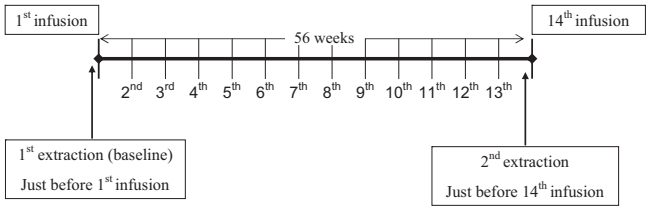

Fig. 1. Schematic of natalizumab administration.

Table 1
Characteristics of patients with relapsing-remitting multiple sclerosis (RRMS). Values are expressed as mean ± S.D. Baseline: patients with multiple sclerosis previous treatment (baseline) and after 56 weeks of treatment with natalizumab (MS-56).

	RRMS (18)
Gender (men/women)	5/13
Age (years)	40.5 ± 7.8 (28–57)
Mean EDSS	
Baseline	4.2 ± 1.5
MS-56	4.4 ± 1.4
Δ (MS-56 – Baseline)	0.2 ± 0.8
Disease duration (years)	7.6 ± 3.9 (3–13)
No. of relapses before treatment	4.6 ± 1.4
No. of relapses during treatment	0.6 ± 0.8
Melatonin by age (pg/ml)	
Baseline	
≤ 40 years	4.4 ± 1.8
> 40 years	7.1 ± 2.2
MS-56	
≤ 40 years	8.1 ± 3.7
> 40 years	17.3 ± 2.2[a]

[a] $P < 0.05$ vs baseline group.

2.2. Statistical analysis

Statistical evaluation was performed using SPSS 17.0® software (SPSS Iberica, Madrid, Spain) for Windows. Intergroup significance was determined by Wilconxon-matched pairs test to analyze nonparametric data. $P < 0.05$ was considered significant.

3. Results

The demographic features of the study groups are presented in Table 1. Our data did not find significant change between EDSS score before and after treatment. However, the EDSS increase suffered by patients after treatment presented a correlation with melatonin levels after treatment (Spearmen Rho, r: 0.777; P=0.04).

Mean serum melatonin pre-treatment levels in the patient group were significantly lower than after the 56 weeks of treatment: 5.0 pg/ml in baseline vs 10.1 pg/ml after 56 weeks of treatment (Fig. 2).

During treatment with natalizumab, women's levels of melatonin in serum increased significantly, 4.3 pg/ml (baseline) vs 10.8 pg/ml (after 56 weeks of treatment). Our data revealed that levels of melatonin increased by 21% in men after natalizumab treatment (Fig. 1). However, the increase caused by nataluzimab was much more intense in women, establishing much higher levels in women than in men (Fig. 2).

When the patients were separated by age into two groups, we only detected significant differences between baseline and after 56 weeks of treatment situation in patients over 40 years (Table 1).

In addition, our results show that serum melatonin elevation is associated with a reduction in oxidative stress markers characterized by an increase in GSH levels and a reduction in 8OHdG levels, whereas PAO did not change (Table 2).

Finally, our analysis found that natalizumab induced a significant reduction in sVCAM (Table 2), as well as these data present a significant correlation between melatonin and sVCAM levels after treatment (Spearman Rho: r −0.479, $P < 0.05$).