Increased interferon-mediated immunity following in vitro and in vivo Modafinil treatment on peripheral immune cells

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A B S T R A C T

The wake-promoting drug Modafinil has been used for treatment of sleep disorders, such as Narcolepsy, excessive daytime sleepiness and sleep apnea, due to its stimulant action. Despite the known effect of Modafinil on brain neurochemistry, particularly on brain dopamine system, recent evidence support an immunomodulatory role for Modafinil treatment in neuroinflammatory models. Here, we aimed to study the effects of in vitro and in vivo Modafinil treatment on activation, proliferation, cell viability, and cytokine production by immune cells in splenocytes culture from mice. The results show that in vitro treatment with Modafinil increased Interferon (IFN)-γ, Interleukin (IL)-2 and IL-17 production and CD25 expression by T cells. In turn, in vivo Modafinil treatment enhanced splenocyte production of IFN-γ, IL-6 and tumor necrosis factor (TNF), and increased the number of IFN-γ producing cells. Next, we addressed the translational value of the observed effects by testing PBMCs from Narcolepsy type 1 patients that underwent Modafinil treatment. We reported increased number of IFN-γ producing cells in PBMCs from Narcolepsy type 1 patients following continuous Modafinil treatment, corroborating our animal data. Taken together, our results show, for the first time, a pro-inflammatory action of Modafinil, particularly on IFN-mediated immunity, in mice and in patients with Narcolepsy type 1. The study suggests a novel effect of this drug treatment, which should be taken into consideration when given concomitantly with an ongoing inflammatory or autoimmune process.

1. Introduction

Modafinil is a psychostimulant drug prescribed for Narcolepsy, excessive daytime sleepiness, shift work and obstructive sleep apnea (Bastuji and Jouvet, 1988; Kuan et al., 2016). Data from clinical and pre-clinical trials indicates that Modafinil antagonizes the norepinephrine transporter (NET) (Madras et al., 2006) and the dopamine transporter (DAT), consequently increasing extracellular dopamine levels in the striatum (Andersen et al., 2010) and nucleus accumbens (Volkow et al., 2009). The D1 and D2 dopamine receptors, as well as the α-1 adrenergic receptor, are essential for mediating the effect of Modafinil on cognition and arousal (Minzenberg and Carter, 2008; Stone et al., 2002).

In addition to neurochemical effects of Modafinil, recent evidence suggests an immunomodulatory activity. In vitro treatment of immortalized microglia cell line with derivatives of Modafinil decreased cyclooxygenase (COX)-2 expression and nitrites production (Jung et al., 2012). On the other hand, in vivo treatment with Modafinil has been showed to improve neuroinflammation induced by methamphetamine administration (Raineri et al., 2012, 2011). Modafinil treatment also prevents the death of dopamine neurons in rats challenged with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a rodent model for Parkinson’s disease (Aguirre et al., 1999; Fuxe et al., 1992; Xiao et al., 2004).

A recent study from our group showed that in vivo Modafinil...
treatment prevented the motor impairment and sickness and depressive behavior induced by a single Lipopolysaccharide (LPS) administration, an effect correlated with the number of CNS-derived macrophages and interleukin (IL)-1β gene expression in brain areas. In addition, the blockage of dopaminergic D1 receptor reversed the Modafinil effects on locomotion and anxiety, but not on depressive-like symptoms and neuroinflammation, indicating that dopamine partially contributes to the modulatory effect of Modafinil (Zager et al., 2017).

Although recent studies pointed to an anti-inflammatory effect of Modafinil treatment in animal models of neuroinflammation, such as induced by LPS (Zager et al., 2017), methamphetamine (Raineri et al., 2012, 2011) or MPTP (Aguirre et al., 1999; Fuxe et al., 1992; Xiao et al., 2004), no study to date have addressed the effects of this drug treatment on peripheral immune function.

Therefore, the primary aim of the present study was to investigate the in vitro effects of Modafinil on cell activation, proliferation, viability, cytokine production and antigen presentation in cultures of primary spleen cells. Secondly, we tested the in vivo effects of the drug by treating mice with either a single or repeated Modafinil administration. Finally, we verified the translational value of the observed effects by testing human peripheral blood mononuclear cells (PBMCs) from patients diagnosed with Narcolepsy type 1. Samples from the same patients were tested before and after continuous Modafinil treatment.

2. Methods

2.1. Animals

Adult C57BL/6J male mice from our own colony, weighing 20–30 g each, were used. The animals were housed in standard polypropylene cages at a controlled room temperature (22 ± 2 °C) and humidity level (65–70%), with artificial lighting (12 h light/12 h dark cycle) and with free access to Nuvilab* rodent chow (Nuvital company, São Paulo, Brazil) and filtered water. Sterilized and residue-free wood shavings were used as animal bedding. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil.

2.2. Drugs

For the in vitro experiments, Modafinil salt was purchased from Sigma Aldrich (USA) and freshly diluted in culture media prior to experiments. For the in vivo treatment, Modafinil (Stavigile, Libbs) tablets were freshly macerated, diluted in β-cyclodextrin solution (Sigma Aldrich, USA; 2:1 drug:β-cyclodextrin), and intraperitoneally administered at the dose of 90 mg/kg (Raineri et al., 2012, 2011; Zager et al., 2017).

2.3. Experiment 1: in vitro experiments

2.3.1. Spleen cell culture and immunophenotyping of cells

Spleens from adult mice (n = 4) were collected, mechanically dissociated, gently passed through a stainless steel mesh and homogenized in 5 ml of sterile DMEM. This suspension was centrifuged at 450g for 5 min and resuspended in sterile ammonium chloride to lyse erythrocytes, following which the solution was centrifuged again. The cells were then resuspended in 5 ml of complete medium (10% fetal bovine serum; PBS), counted, adjusted to a concentration of 5 × 10^6 cells and seeded in 96-well plates. Total splenocytes were incubated with Modafinil at different concentrations (0.03, 0.1, 0.3, 1, 3 and 10 mM) for 30 min, followed by anti-CD3 (1 μg/ml; Biolegend, USA) for T cell stimuli, and with Lipopolysaccharide (LPS; 1 μg/ml) for antigen presenting cells stimuli. After 24 h of incubation at 37 °C (5% CO_2), cultured plates were centrifuged at 450 g for 5 min and the supernatants were then collected and frozen at −80 °C until the analysis.

The cultured cells were resuspended and centrifuged at 450 g for 5 min. The cells stimulated with anti-CD3 were labeled with antibodies against CD4, CD62L and CD25 (BD Pharmingen, USA), whereas the cells stimulated with LPS were labeled with antibodies against CD11b and IAß (BD Pharmingen, USA). After incubation for 30 min at room temperature in the dark, cells were rinsed twice and resuspended in 100 μl of PBS. The single cells were immunophenotyped by analyzing the frequency and mean intensity of fluorescence markers in a FACS Accuri flow cytometer (BD Immunocytometry Systems, San Jose, USA).

2.3.2. T cell proliferation assay

Total splenocytes were incubated with carboxyfluorescein succinimidyl ester (CFSE) for 15 min at 37 °C. After CFSE incubation, cells were seeded in 96 well plates and Modafinil was added at different concentrations (0.03, 0.1, 0.3, 1, 3 and 10 mM) for 30 min, followed by anti-CD3 (1 μg/ml). After 72 h of incubation at 37 °C (5% CO_2), cultured plates were centrifuged at 450 g for 5 min and resuspended in PBS with anti-CD4 antibody (BD Pharmingen, USA). After incubation for 30 min at room temperature in the dark, cells were rinsed twice and resuspended in 100 μl of PBS. The single cells were immunophenotyped by analyzing the frequency and mean intensity of fluorescence markers in a FACS Accuri flow cytometer, using the FITC channel for the CFSE expression (BD Immunocytometry Systems, San Jose, USA).

2.3.3. Cell death experiment

Total splenocytes were isolated and cultured as described previously, and cells were cultured with Modafinil in the following concentrations: 0.03, 0.1, 0.3, 1, 3 and 10 mM for 24 h. At the end of this period, cells were incubated with FITC-conjugated Annexin-V (BD Pharmingen, USA) and propidium iodide (PI) for 15 min at room temperature in the dark. Cells were analyzed by the frequency of fluorescence markers in a FACS Accuri flow cytometer (BD Immunocytometry Systems, San Jose, USA).

2.4. Experiment 2: in vivo experiments

2.4.1. Experimental protocol

The animals (n = 6/group) were intraperitoneally treated with Vehicle or Modafinil (Chronic Modafinil group) for 5 consecutive days. An extra group of animals received a single Modafinil administration (Acute Modafinil group). All drug treatments were performed between 08:00 and 12:00. Thirty minutes after the last drug administration, mice were decapitated, the blood was collected in tubes with heparin and spleens were surgically removed under sterile conditions.

2.4.2. Spleen cell culture and phenotyping of cells

Total splenocytes were incubated with anti-CD3 (1 μg/ml) for T cell stimuli, and with LPS (1 μg/ml) for antigen presenting cells stimuli. After 24 h of incubation at 37 °C (5% CO_2), cultured plates were centrifuged at 450 g for 5 min and the supernatants were collected and then frozen at −80 °C until the analysis. The cells stimulated with anti-CD3 were labeled with antibodies against CD4, CD62L and CD25 (BD Pharmingen, USA), whereas the cells stimulated with LPS were labeled with antibodies against CD11b and IAß (BD Pharmingen, USA). The single cells were immunophenotyped by analyzing the frequency and mean intensity of fluorescence markers in a FACS Accuri flow cytometer (BD Immunocytometry Systems, San Jose, USA).

2.4.3. Oxidative burst and phagocytosis by circulating neutrophils

Briefly, 100 μl of whole blood (2 × 10^6 cells/100 μl) was mixed with 200 μl of dichlorofluorescin (DCFH; 0.3 mM) in phosphate-buffered saline (PBS) and 100 μl of either Propidium iodide-labeled S. aureus (SAPI, 2.4 × 10^9 bacteria/ml) or phorbol myristate acetate (PMA/100 ng) in different tubes. Samples were incubated at 37 °C for 30 min. Phagocytosis reactions were stopped by adding 2 ml of cold EDTA solution (3 mM). After centrifugation (250 g for 10 min), the
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