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Effects of minocycline add-on treatment on brain morphometry and cerebral perfusion in recent-onset schizophrenia

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ABSTRACT

Increasing evidence suggests that the tetracycline antibiotic minocycline has neuroprotective effects and is a potential treatment for schizophrenia. However, the mechanisms of action of minocycline in the CNS remain elusive. The aim of this study was to investigate the effects of minocycline on brain morphology and cerebral perfusion in patients with recent-onset schizophrenia after 12 months of a randomized double-blind, placebo-controlled clinical trial of minocycline add-on treatment. This study included 24 outpatients with recent-onset schizophrenia randomized for 12 months of adjuvant treatment with minocycline (200 mg/d) or placebo. MRI (1.5 T) and [^{99m}Tc]-ECD SPECT brain scans were performed at the end of the 12-month of trial. Between-condition comparisons of SPECT and MRI brain images were performed using statistical parametric mapping and analyzed by voxel-based morphometry (VBM). Minocycline adjuvant treatment significantly reduced positive and negative symptoms when compared with placebo. The VBM analysis of MRI scans showed that the patients in the placebo group had significant lower gray matter volumes in the midposterior cingulate cortex and in the precentral gyrus in comparison with the patients in the minocycline group. In addition, a decreased ECD uptake in the minocycline condition was observed in fronto-temporal areas. These results suggest that minocycline may protect against gray matter loss and modulate fronto-temporal areas involved in the pathophysiology of schizophrenia. Furthermore, minocycline add-on treatment may be a potential treatment in the early stages of schizophrenia and may ameliorate clinical deterioration and brain alterations observed in this period.

1. Introduction

Despite the great variety of antipsychotics currently available, longitudinal cohort studies with patients in their first episode of psychosis have shown aggravation in several psychopathological domains (McGlashan, 1998; Hoff et al., 1999; Lieberman, 1999; Stirling et al., 2003), as well as progressive gray matter loss and altered brain function, particularly in fronto-temporal areas (Lieberman, 1999; Cahn et al., 2002; Bachmann et al., 2004; Perez-Neri et al., 2006; Whitford et al., 2006; Koo et al., 2008; van Haren et al., 2008; Mane et al., 2009; Smieskova et al., 2009).

Increasing evidence points out that minocycline, a broad-spectrum tetracycline antibiotic, has neuroprotective effects in different neurological conditions (e.g., brain ischemia) (Domercq and Matute, 2004; Kim and Suh, 2009). Translational neuroscience data from both animal and human studies have shown that minocycline is a potential treatment for schizophrenia. In three studies with animal models of psychosis, the treatment with minocycline prevented or reversed the behavioral effects of administration of NMDA antagonists (Levkovitz et al., 2007;

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Zhang et al., 2007; Fujita et al., 2008). Subsequently, a case report and an open study with the addition of minocycline to the usual antipsychotic treatment of patients with schizophrenia showed significant improvement in positive, negative and cognitive symptoms (Miyaoka et al., 2007, 2008). Adjunctive minocycline to clozapine was also effective in improving positive and negative symptoms of two super-refractory patients (Kelly et al., 2011). Additionally, a randomized double-blind placebo-controlled clinical trial of minocycline add-on treatment showed an improvement in negative and cognitive symptoms in patients with schizophrenia (Levkovitz et al., 2010), and another randomized double-blind placebo-controlled clinical trial demonstrated an improvement mainly in the negative symptoms (Chaudhry et al., 2012).

In spite of these findings, the effects of minocycline in the central nervous system remain elusive and have not been systematically investigated with neuroimaging techniques. The hypothesis of this study is that minocycline add-on treatment may improve diverse psychopathological domains of schizophrenia and prevent brain alterations (especially in fronto-temporal areas) that usually occur in the early course of schizophrenia. Hence, the aim of this study was to investigate the effects of minocycline on brain morphology and cerebral perfusion in patients with recent-onset schizophrenia after 12 months of a randomized double-blind, placebo-controlled clinical trial of minocycline added to the treatment as usual.

2. Methods and materials

2.1. General context of the study

This research focused on the neuroimaging findings of a randomized double-blinded placebo-controlled study of minocycline add-on treatment. The clinical data of this study have already been published as part of a multi-site clinical trial that included patients from Brazil and Pakistan (Chaudhry et al., 2012). Hence, the aim of this study was to investigate the effects of minocycline on brain morphology and cerebral perfusion using MRI and ^{99m}Tc-ECD SPECT scans of the patients followed at the Brazil site after 12 months of the addition of minocycline or placebo to regular antipsychotic treatment. The trial was registered at ClinicalTrials.gov (Identifier: NCT00916461).

2.2. Procedures

This study included 30 patients with DSM-IV diagnosis of schizophrenia with up to five years of illness and with stable antipsychotic dose for at least four weeks before the first study evaluation. Subjects were selected from among patients followed up in the Hospital of Ribeirão Preto Medical School (University of São Paulo). The patients and relatives signed an informed consent. This study obtained ethical approval at the local research ethics committee. Patients were randomized to take minocycline (200 mg/d) or placebo in a follow-up of 12 months. Placebo pills had organoleptic aspect and packaging that were identical to the minocycline ones. Both minocycline and placebo pills were provided by "Stiefel Laboratories Inc." Clinical ratings were evaluated by the Clinical Global Improvement (CGI) and the Positive and Negative Syndrome Scale (PANSS) and its subscales. Side effects were assessed by the UKU side effects scale and the Abnormal Involuntary Movements Scale (AIMS). The procedures of the clinical trial have been previously fully described in Chaudhry et al. (2012). The patients underwent both MRI and SPECT brain scans after 12 months of minocycline or placebo add-on treatment.

2.3. Structural MRI acquisition, processing and analysis

MRI scans were obtained by a SIEMENS MRI scanner with a main magnetic field of 1.5 T. Slices were acquired along the entire brain, always using the same standardized protocol, optimized to allow good signal differentiation between areas of gray matter and white matter. The protocol consisted of a SPGR sequence (TR = 9.7 ms, TE = 4 ms, flip angle = 12, FOV = 35 mm, 265 × 265 matrix) in sagittal sections with a resolution of 1 mm^3/voxel.

Voxel-based Morphometry (VBM) analysis of the images was made with the SPM2 (Statistical Paremetric Mapping) running on MATLAB software version 7.0 (MathWorks, Natick, MA, USA). The images originally acquired in DICOM format were converted to Analyze format (compatible with SPM2) through the software MRIcro (http://www.sph.sc.edu/comd/rorden/mricro.html). Image processing followed the optimized protocol for VBM described by Good et al. (2001).

Initially, the anterior commissure was manually demarcated and the images were subsequently reoriented to the neurological pattern. From this step, as described in Good et al. (2001), an a priori T1 template and specific templates for gray matter were specifically created for this study. The original images were then normalized based on a previously established T1 template, using linear and nonlinear transformations (the latter being made with functions of 7 × 8 × 7 basis and 12 iterations). The normalized images were segmented and the gray matter (and its specific template previously created) from the resulting components were utilized to obtain normalization parameters to be applied in the original images. These optimally normalized images were segmented and subsequently modulated by the Jacobian determinant (extracted from the normalization parameters previously obtained) and smoothed with a 12 mm full-width at half maximum (FWHM) isotropic Gaussian kernel.

In order to show local differences in gray matter, between-group comparisons of brain volumes were made by using the function "single subject: conditions and covariates", with the total intracranium volume (ICV) as a confounding variable. Resulting statistics at each voxel were transformed into Z scores, limited in Z = 3.09 (corresponding to $p < 0.001$, uncorrected for multiple comparisons; one-tail) and only clusters with more than 25 voxels were considered, visually presented as three-dimensional statistical parametric maps (SPM) in standard space (Talairach and Tournoux, 1988). The threshold of $p < 0.001$ (uncorrected for multiple comparisons) have been used in previous neuroimaging studies that used SPM analyses (Blackwood et al., 1999; Bremner et al., 1999; Dougherty et al., 1999; Busatto et al., 2000; Valente et al., 2005; Crippa et al., 2011) and has been empirically indicated to provide good protection against false positive results when there are clear hypotheses to the location of findings. These SPM maps were inspected for the existence of clusters of significant between-group differences in brain regions that have been predicted a priori (fronto-temporal cortices and cingulate gyrus). The resulting coordinates in the MNI stereotactic space (Montreal Neurological Institute, Canada) were converted to Talairach space (Talairach and Tournoux, 1988), using the transformation suggested online (NEUROVIA, University of Minnesota, Minneapolis VA Medical Center, http://www.neurovia.umn.edu/cgi-bin/tal_atlas). After image conversion, the software Talairach Deamon (Lancaster et al., 2000) was used to finally obtain the location description.

2.4. SPECT image acquisition, processing and analysis

The acquisition of SPECT images started 20 min after an intravenous injection of 740 MBq (20 mCi) of ^{99m}Tc-ECD at rest, using a Sopha® DST (Sophy Medical Vision, Twinsburg, USA) dual-detector gamma camera. Collimators for low energy and high resolution were used, with 128 views in a circular orbit of 360°, acquired in a matrix of 128 × 128 (30 s per view) and with total acquisition time of 30 min and approximately 75,000 counts per frame/head. Raw images were prefiltered with a Butterworth filter (order 9, cutoff frequency of 0.14) and were reconstructed in axial (orbito-meatal line) and parallel planes to the long axis of the temporal lobe, from which coronal images were produced. Attenuation correction was carried out considering a pixel size of 2.55 mm and using the method of Chang's first order (coefficient 0.12/cm).