NeuroImage: Clinical 4 (2014) 454–460

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Neuroprotective effects of testosterone treatment in men with multiple sclerosis

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ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 3 March 2014
Accepted 4 March 2014

ABSTRACT

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease of the central nervous system. While current medication reduces relapses and inflammatory activity, it has only a modest effect on long-term disability and gray matter atrophy. Here, we have characterized the potential neuroprotective effects of testosterone on cerebral gray matter in a pilot clinical trial. Ten men with relapsing–remitting MS were included in this open-label phase II trial. Subjects were observed without treatment for 6 months, followed by testosterone treatment for another 12 months. Focal gray matter loss as a marker for neurodegeneration was assessed using voxel-based morphometry. During the non-treatment phase, significant voxel-wise gray matter decreases were widespread ($p \leq 0.05$ corrected). However, during testosterone treatment, gray matter loss was no longer evident. In fact, a significant gray matter increase in the right frontal cortex was observed ($p \leq 0.05$ corrected). These observations support the potential of testosterone treatment to stall (and perhaps even reverse) neurodegeneration associated with MS. Furthermore, they warrant the investigation of testosterone's neuroprotective effects in larger, placebo controlled MS trials as well as in other neurodegenerative diseases. This is the first report of gray matter increase as the result of treatment in MS.

1. Introduction

Multiple sclerosis (MS) is a putative autoimmune disease characterized by a relapsing–remitting disease course leading to progressive disability, inflammation, and neurodegeneration (Vigeveno et al., 2012). MS relapses are associated with inflammation and the development of white matter lesions. As a result, anti-inflammatory treatments have been developed based on their ability to reduce relapses and white matter lesions. However, inflammatory activity and white matter lesion burden are only weakly correlated with clinical disease progression (Brex et al., 2002). Gray matter (GM) atrophy correlates strongly with clinical disability (Ontaneda et al., 2012; Rudick and Trapp, 2009; Vigeveno et al., 2012) and neurodegeneration in MS (Bo, 2009; Gold and Voskuhl, 2009; Rudick and Trapp, 2009; Vigeveno et al., 2012). This atrophy is evident from the earliest stages of disease, even before a clinically definite diagnosis can be made (Dalton et al., 2004) and continues throughout the disease course (Fisher et al., 2008). Consequently, GM atrophy has been suggested as a surrogate marker for disease progression and neurodegeneration in MS (Grassiot et al., 2009). GM atrophy has produced different results than inflammatory markers as outcome measures in clinical trials: while anti-inflammatory treatments in MS have been shown to reduce the occurrence of inflammatory markers such as new white matter lesions or relapse rates, their effects on GM atrophy and permanent disability have been modest (Hardmeier et al., 2005; Miller et al., 2007; Rao et al., 2002). To effectively target gray matter atrophy and diminish or prevent permanent disability in MS, neuroprotective therapies are needed.

Testosterone has been shown to be neuroprotective in animal studies (Gold and Voskuhl, 2009; Hussain et al., 2013) including the most widely used MS model, experimental autoimmune encephalomyelitis (EAE) (Bebo et al., 1999; Dalal et al., 1997; Ziehn et al., 2012). Analogously, we hypothesized that testosterone treatment in human disease may be neuroprotective and this would be reflected

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2213-1582/$ - see front matter
http://dx.doi.org/10.1016/j.nicl.2014.03.001

as a slowing of gray matter atrophy. Thus, the aim of the current study was to evaluate the effects of testosterone treatment on local changes in gray matter volume in MS. Changes in local gray matter volume were quantified using voxel-based morphometry (VBM), a sophisticated, objective whole-brain analysis technique (Draganski et al., 2004). Ten male patients with MS were enrolled in an open-label phase II clinical trial. The patients were observed prior to treatment for 6 months (observation phase), followed by a 12-month period of treatment with testosterone. To account for "wash-in effects", this 12-month treatment period was divided into an initial 6-month transition phase, which allowed the drug to take action, followed by a 6-month protection phase. Statistically significant changes in GM concentration were mapped for each phase and the GM volume of these localized changes was plotted as a percent change. In addition, the annualized whole-brain GM atrophy rate was calculated in a supplementary analysis.

2. Material and methods

2.1. Study design and subjects

Participants were eligible if they met the criteria for clinically definite relapsing–remitting MS, had had at least one clinical relapse or the appearance of at least one enhancing lesion on MRI over the preceding two years, but were not receiving disease-modifying treatment. The original study has been described in detail (Sicotte et al., 2007). Briefly, this study was an open label phase II trial to assess the safety and tolerability of testosterone treatment using 10 g of gel containing 100 mg of testosterone (Androgel) applied topically daily for one year. The ten men enrolled had a median Expanded Disability Status Scale (EDSS) score of 2.0 (range: 1.5–2.5), a median disease duration of 12.5 years (range: 0.5–25.0 years), and a mean age of 46 years (range: 29–61 years). A cross-over trial design was chosen, where subjects served as their own controls. The protocol was approved by the University of California Los Angeles (UCLA) Human Subjects Protection Committee and the institutional review board of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

Contrast-enhanced, FLAIR, and high-resolution T1-weighted brain MRIs were obtained every month from baseline (month 0) until the end of the trial (month 18). All ten subjects completed all nineteen monthly scans (baseline plus monthly scans for 18 months) and were included in the current analysis. Testosterone treatment started at the end of month six. To allow the drug enough time to work ("wash-in"), we divided the trial into three parts: an untreated observation phase, a transition phase, and a protection phase. The observation phase was between baseline and end of month six. The transition phase was defined as end of month six through end of month twelve, leaving the last six months of the trial as the protection phase.

2.2. Image acquisition and processing

Magnetic resonance imaging (MRI) data was acquired on a 1.5 T Siemens Sonata scanner using a T1-weighted sequence (MPRAGE) with the following parameters: TR = 1900 ms, TE = 4.38 ms, flip angle = 15°, 128 sagittal slices, matrix: 256 × 256, and voxel dimensions: 0.9375 × 0.9375 × 1.2 mm^3. In addition, a Fluid Attenuated Inversion Recovery (FLAIR) sequence was obtained during the same session using the following parameters: TR = 9140 ms, TE = 100 ms, flip angle = 180°, 50 axial slices, matrix: 256 × 256, and voxel dimensions: 0.9375 × 0.9375 × 3 mm^3. Brain images were processed and examined using SPM8, the LST toolbox, and the VBM8 Toolbox following previously described methods (Luders et al., 2009; Schmidt et al., 2012). This processing consisted of lesion in-painting to prevent a possible confound of white matter lesions on tissue segmentation, followed by tissue-segmentation and normalization to a common reference space that allows for voxel-wise testing. Briefly, white matter lesions were automatically delineated using a lesion-growing algorithm (Schmidt et al., 2012) that used information from both the FLAIR and T1-weighted images simultaneously. The lesion growing algorithm was validated and fine-tuned using manual delineations of lesions as described (Schmidt et al., 2012). Based on these delineations, the lesions were in-painted as white matter in the T1-weighted scans (Chard et al., 2010; Schmidt et al., 2012) and the in-painted images were quality controlled to assure accuracy. To accommodate for the longitudinal design of this study, the lesion in-painted images were subsequently realigned for each subject using half-way registrations and corrected for bias-field inhomogeneities using the VBM8 Toolbox (see http://dbm.neuro.uni-jena.de/vbm8/VBM8-Manual.pdf).

Further preprocessing with this toolbox included tissue-classification into gray matter, white matter, and cerebrospinal fluid followed by a registration to MNI space using linear and non-linear transformations (Luders et al., 2009; Luders et al., 2013). More specifically, the tissue segmentation algorithm accounted for partial volume effects (Tohka et al., 2004), and was based on adaptive maximum a posteriori estimations (Rajapakse et al., 1997), a spatially adaptive non-local means denoising filter (Manjon et al., 2010), as well as a hidden Markov random field model (Cuadra et al., 2005). This tissue classification was independent of tissue probability maps (Luders et al., 2009; Luders et al., 2013), thus acting as an additional safeguard against a potential influence of lesions and altered geometry. Using affine registration and the non-linear DARTEL algorithm (Ashburner, 2007), the individual GM and WM segments in native-space were then normalized to the DARTEL-Template supplied with the VBM8 Toolbox (see http://http://dbm.neuro.uni-jena.de/vbm). This allowed for a comparison between time-points and subjects on voxel-level, yielding an extremely high regional specificity. A quality check was performed using tools from the VBM8 Toolbox and individual visual assessment, which yielded no artifacts or failed segmentation/normalization of the data. Finally, the gray matter segments were smoothed with a Gaussian kernel (12 mm full width at half maximum). These smoothed gray matter segments constituted the input for the statistical model. For visualization, a mean template from all subjects was created using normalized whole-brain images. This way, significant results from the statistical analysis were directly superimposed on the subjects' mean anatomy for anatomic localization of significant changes in local gray matter volume.

2.3. Statistical analyses

The statistical model included all ten subjects and their smoothed gray matter segments from four time points: at baseline, after 6 months (i.e. at the end of the observation phase), after 12 months (i.e. at the end of the transition phase), and after 18 months (i.e. at the end of the protection phase). This selection allowed us to investigate the voxel-wise volume changes over the three phases of the trial. Specifically, a "subject × condition" model was generated (with condition being the four time points). In this model the inter-individual differences between subjects were modeled by the subject factor, while changes between the time points were modeled for each subject by the condition factor. To control for false positives, threshold-free cluster enhancement (TFCE) (Smith and Nichols, 2009) in conjunction with family-wise error correction was used to detect significant clusters at $p \leq 0.05$.

Patterns of significant local change (i.e. significant gray matter decrease and increase) were visualized on a series of maximum intensity projections illustrating significant changes during the observation, transition, and protection phases. In a subsequent step, the exact locations of gray matter change during the observation and protection phases were mapped on the mean template, as described above. The time course of the volumetric changes for each significance cluster was extracted over all eighteen months of the trial and plotted as percent change to better assess the effects of testosterone therapy on