Lower brain-derived neurotrophic factor in serum of relapsing remitting MS: Reversal by glatiramer acetate

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Received 4 April 2005; accepted 6 July 2005

Abstract

Neuronal growth factors may exert a neuroprotective effect in multiple sclerosis (MS). We found reduced levels of brain-derived neurotrophic factors (BDNF) in the serum and CSF of relapsing–remitting MS patients, which was reversed by therapy with glatiramer acetate. BDNF may play a protective role in MS, and immunomodulation therapy, such as with glatiramer acetate, may enhance the action this mechanism.

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Keywords: Multiple sclerosis; Neuroprotection; Brain-derived neurotrophic factor; Glatiramer acetate

1. Introduction

Multiple sclerosis (MS) is a multifocal disease of the CNS white matter, in which inflammatory foci are associated with demyelination and axonal damage (Brosnan and Raine, 1996). Regardless of the order of the mechanisms by which these features appear, it is well established that the extent of axonal and neuronal loss correlates with the degree of disease disability. Therefore, it is essential to search for possible neuroprotective and neuroregenerative mechanisms.

Previously identified neurotrophic factors can be grouped into families on the basis of their peptide structure and their mechanism of action. The characterized families correspond to the neurotrophin family, the glial cell-derived neurotrophic factor family and the neuropoietic cytokines. The neurotrophin family comprises the nerve growth factor (NGF), the brain-derived neurotrophic factor (BDNF), neurotrophin 3 and neurotrophin 4/5 (Vega et al., 2003). These factors play a role not only in the normal ontogenetic development of the embryonic and immature nervous system, but are also reactivated following CNS damage, playing an important role in promoting neuronal repair and protection (Connor and Dragunow, 1998). They have, therefore, been proposed as therapeutic strategies in neurodegenerative and inflammatory diseases of the CNS, such as MS.

BDNF was found to be present in immune cells and astrocytes of MS lesions (Stadelmann et al., 2002). Immune cells such as those that infiltrate the demyelinating plaques produce BDNF (Kerschensteiner et al., 1999), (Besser and Wank, 1999). It has been proposed that the immune response in MS may exert a neuroprotective effect that is mediated via neurotrophic factors secretion, mainly by BDNF (Stadelmann et al., 2002), (Hohlfeld et al., 2000).

Recent studies found that stimulation of T cells with GA induced an increased production of BDNF, and therefore suggest that therapeutic GA may promote immune-mediated neuroprotection in MS (Ziemssen et al., 2002). Furthermore, in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, GA-specific cells that accumulated in the brain strongly expressed BDNF while non-GA-specific cells only marginally expressed BDNF (Aharoni et al.,...
2003). It should be noted that GA was found to reduce surrogate markers for tissue damage, as evidenced by brain MRI (Rovaris et al., 2001), (Ge et al., 2000).

In this study, we examined BDNF levels in the serum and CSF of patients with relapsing–remitting MS, in non-treated MS patients and matched patients that are treated with disease-modifying therapies and healthy controls.

2. Methods

After signing informed consent forms, 74 patients with definite relapsing–remitting MS according to poser’s criteria and 28 aged-matched apparently healthy individuals (controls) donated blood for the purposes of this study. Twenty-six patients were being treated with interferon-β and 27 patients with glatiramer acetate, while 21 had received no treatment. The 53 treated patients had received the specified medication for at least 6 months before study entry and had not undergone any additional immune-related therapy for at least 6 months (Table 1). CSF samples were donated by nine non-treated MS patients and by seven patients with other neurological diseases (four were investigated for pseudotumour cerebri and three for inflammatory polyneuropathy). Serum was extracted by standard centrifugation, and CSF was taken only from patients whose neurological evaluation indicated the need for a spinal tap. Supernatants of PBMCs stimulated with anti-human CD3 monoclonal antibody (PharMingen, San Diego, CA, USA) were collected after 24 h of incubation (37 °C, 5% CO2).

The levels of BDNF in the serum CSF and supernatants were studied by a Dou Set ELISA kit for human BDNF (R&D Systems, Minneapolis, MN, USA) and a Termo Max ELISA reader (Molecular Devices microplate reader, USA).

BDNF production in various immune cells, such as T cells, monocytes and B cells, was studied by flow cytometry and fluorochrome-conjugated monoclonal antibodies against CD3, CD4, CD8, CD14 and CD19 (PharMingen) as well as by intracytoplasmic staining for BDNF as described elsewhere (Aharoni et al., 2003).

All the data are presented as mean ± standard deviation. Statistical analyses for comparing the BDNF levels between the study groups were carried out by Student’s t test.

3. Results

We first compared the mean serum levels of BDNF of untreated patients with relapsing–remitting MS with those of the age-matched controls and found that the former was significantly less (44.7 ± 16.8 ng/ml) than the latter (60.7 ± 23.9 ng/ml, p = 0.013, Fig. 1a). Assuming that the reduced

<table>
<thead>
<tr>
<th>Participants</th>
<th>Serum donors (n)</th>
<th>Age (y)</th>
<th>Female: male</th>
</tr>
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<tbody>
<tr>
<td>All patients with multiple sclerosis</td>
<td>74</td>
<td>34.2±12.4</td>
<td>48:26</td>
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<tr>
<td>Untreated patients</td>
<td>21</td>
<td>31.1±8.1</td>
<td>13:8</td>
</tr>
<tr>
<td>Interferon-β-treated patients</td>
<td>26</td>
<td>35.5±9.6</td>
<td>18:8</td>
</tr>
<tr>
<td>Glatiramer acetate-treated patients</td>
<td>27</td>
<td>35.3±11.1</td>
<td>17:10</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>28</td>
<td>32.9±6.4</td>
<td>18:10</td>
</tr>
</tbody>
</table>

Fig. 1. ELISA analysis of serum and CSF levels. (a) Untreated patients with relapsing–remitting MS had significantly reduced brain-derived neurotrophic factor (BDNF) serum levels compared with healthy controls. (b) Comparison between times of relapse to times of remission showed a trend toward reduction of BDNF during relapses. (c) The CSF levels of BDNF were also reduced in patients with MS compared to patients with other neurological diseases (OND). (d) Therapy with glatiramer acetate (GA) but not with interferon-β (IFN b) normalized the serum levels of BDNF. Significant differences (p < 0.05) are labeled with asterisks.
BDNF level may be related to disease activity, we compared the levels between time of relapse to time of remission in the untreated patients group. A cross-sectional comparison of serum BDNF levels between samples that were taken during relapses (n = 9) and those during remission (n = 15) failed to reveal any differences (45.1 ± 22.3 ng/ml and 44.4 ± 13.9 ng/ml, respectively). When the comparison between time of relapse to time of remission was made longitudinally in the same patients, we found a trend towards reduction of the levels during relapses, as in 6 out of 7 patients the serum BDNF levels were lower than that during remission (Fig. 1b). Similarly, when we studied the levels of BDNF in CSF, we found reduced levels among MS patients during relapse compared to patients with other neurological diseases (141.3 ± 23.4 pg/ml versus 160.9 ± 3.8 pg/ml, respectively; p = 0.047, Fig. 1c).

We then studied the effect of the two type of therapies on the concentrations of BDNF in serum (Fig. 1d) and found that the levels of patients treated with GA (59.9 ± 20.2 ng/ml) were similar to those of the controls and significantly higher than both those of untreated patients (44.7 ± 16.8, p = 0.006) and of patients who were treated with interferon-β (49.1 ± 19.7, p = 0.041). In addition, the BDNF values in patients who were treated with interferon-β were higher than those of the controls, but not to a level of statistical significance (p = 0.733). We then further studied the effect of GA on immune cells and found that stimulated PBMCs of patients who were treated with GA secreted significantly higher levels of BDNF (n = 8, 1859 ± 614 pg/ml) compared to the BDNF levels of untreated patients (n = 11, 973 ± 395 pg/ml, p = 0.013). In order to analyze the profile of BDNF production by cells that comprise the PBMCs, we studied the intracytoplasmic production of BDNF in stimulated PBMCs of three healthy donors and found BDNF-positive cells in 28.0% of the monocytes (CD14+ cells), 1.46% of CD4+ T cells, 0.2% of CD8+ T cells and 0.8% of B cells (CD19+ cells).

4. Discussion

It has been established that BDNF has a protective and regenerative effect on injured nerve cells and that it is produced by immune cells as well as by other cells of visceral organs that may play a role in tissue repair in MS (Vega et al., 2003), (Connor and Dragunow, 1998), (Kerschensteiner et al., 1999), (Lommatzsch et al., 1999). We believe that our current study is the first to show that BDNF levels are reduced in the serum and CSF of patients with relapsing–remitting MS, and therefore support that notion that BDNF participates in protective mechanisms against tissue damage in MS. The reduced BDNF levels suggest that there is a reduction in tissue protection by BDNF in relapsing–remitting MS or, alternatively, that there is an increase in the consumption of BDNF by the CNS due to the damaged tissue in MS. Given that BDNF may play a role in the protection or regeneration of brain tissue of patients with MS, a therapeutic approach that would increase its production may help to slow down or even halt the accelerating axonal loss in MS. Our results showed that GA may have such an effect by demonstrating normalization in the serum levels of patients who were treated with it compared to other patients with relapsing–remitting MS. This effect of GA on serum levels of BDNF can be explained, at least partially, by our observation of increased secreted levels of BDNF from PBMCs of GA-treated patients and by the effect of GA-specific T cells on the production of BDNF (Ziemssen et al., 2002), and this raises the possibility that such a BDNF-promoting effect on the part of GA may exist in other immune and non-immune types of cells. GA is known to induce immune deviation from T helper-type 1 to T helper-type 2 response within and outside the CNS (Neuhaus et al., 2000): the suggested neuroprotective effect of GA is proposed as being another component of its therapeutic effect on the immune system.

Acknowledgment

Esther Eshkol is thanked for editorial assistance.

References


