Immunologic Aspects of Topiramate Treatment in West Syndrome Patients

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Abstract

Objective: To explore the regulatory effect of topiramate on immunological functions in patients with West syndrome (WS).

Methods: Flow cytometric measurement of the proportion of lymphocyte subsets in peripheral blood and nephelometric measurement of the immunoglobulins IgG, IgM and IgA in the serum of 8 patients with WS and of 25 normal healthy infants before and after topiramate treatment.

Results: CD4+ T cells and serum levels of IgG were increased in untreated WS patients and normalized after topiramate treatment. In contrast, natural killer cells were decreased in untreated WS patients, but increased to control levels by topiramate treatment. B cells and serum IgA levels were decreased by topiramate treatment. No significant changes were seen in CD8+ T cells and IgM serum levels of WS patients neither in comparison to controls nor in response to topiramate treatment.

Conclusions: Topiramate normalizes altered immunological functions of patients with WS.

Key words: Epilepsy; Immunological function; Topiramate; West syndrome

Introduction

West syndrome (WS) is an age-specific epileptic syndrome with onset in infancy characterized by clusters of epileptic spasms, a peculiar interracial EEG pattern of hypersrrhythmia, and mental deterioration. It may result from different neurological disorders, but often an etiological explanation cannot be identified. Although there are only few data on immune cell functions in epilepsy available so far,1 growing evidence suggests that autoimmune mechanisms might be involved in the pathogenesis of WS. A study of Mota et al.2 demonstrated high serum levels of precipitating antibodies to a saline extract of brain tissue in 15 patients with WS. Haraldsson et al.3 found higher levels of IgG and IgM in the serum of children with cryptogenic WS and other forms of childhood epilepsy compared to healthy control children. Levels of interleukin (IL)-2, tumor necrosis factor (TNF)-α and interferon (IFN)-α were significantly higher in both cryptogenic and symptomatic WS groups than in a control group according to a study of Liu et al.4 Successful treatment of WS with steroids and intravenous immunoglobulins5,6 also suggested that an autoimmune mechanism might play a causative role for WS.

A new anti-epileptic drug with proven efficacy against spasms in WS was topiramate.7,8 It appeared to have multiple mechanisms of action, including a state-dependent blockade of sodium channels, antagonism of glutamate, and potentiation of GABA-mediated neurotransmission.7 However, it is not known, whether the drug is also affecting the immune response in WS patients. We have, therefore, studied humoral and cellular immune parameters in WS
patients before and after topiramate treatment in comparison to normal healthy children.

Materials and Methods

Study Subjects

Eight patients with newly diagnosed and previously untreated WS entered the study in the Pediatric Neurology Department of Beijing Children Hospital. The age of the patients was ranging from 6 to 13 months. All patients were diagnosed according to the international criteria.9 Complete blood counts and routine biochemical and urinary analyses were performed in addition to electroencephalographic recordings. All patients treated with topiramate were free of seizures for at least 2 months. First blood samples were taken before therapy and second blood samples were taken after treatment with topiramate for a period of 2 months without spasms. Twenty-five healthy control children of similar age were investigated with repeated sampling in the same way.

Surface Immunofluorescence Staining and Flow Cytometric Analysis

Peripheral blood mononuclear cells were isolated as previously described.10 The cell numbers in the suspension were adjusted to 1 x 10^6 viable cells/ml medium (Flow Lab, Irvine, UK). The cells were further cultured in 6-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) at 37°C for 1 h. The adherent cells were collected and stained with phycoerythrine (PE)-labeled anti-human CD3, PE-labeled anti-human CD19, fluorescein isothiocyanate (FITC)-labeled anti-human CD4, FITC-labeled anti-human CD8, and FITC-labeled anti-human NK (CD4-FITC/CD3-PE, CD8-FITC/CD3-PE, and CD(16+56)-FITC/CD3-PE), respectively. PE- and FITC-labeled isotype-matched immunoglobulins served as control antibodies. All conjugated antibodies were purchased from Pharmingen (San Diego, CA). Staining was performed by antibody incubation at 4°C for 1 h. After washing with 1% bovine serum albumin in phosphate buffered saline, cells were analyzed in a FACScan using CellQuest software (both from Becton Diskinson, Mountain View, CA). Results are given as percentages of lymphocyte subsets out of total peripheral mononuclear blood cells.

Determination of IgG, IgM, and IgA in Serum

Serum test samples were collected in routine manner used for any clinical laboratory test. Program Auto ICS was applied for nephelometric determination of serum IgG, IgM and IgA according to the manufacture’s instruction (Array™ 360, Beckman Instruments, Galway, Ireland).

Statistics

Differences between pairs of groups were tested by Student’s t-test and Mann-Whitney’s U-test. Differences between the different groups were evaluated by the Kruskal-Wallis one way analysis of variance (ANOVA). The level of significance was set to p<0.05. All significance tests were two-sided. All calculations were performed with InStat statistical analysis software (GraphPad Software, San Diego, CA).

Ethics

Healthy control children were approved by their parents and YAN-QING Research Human Ethics Committee Beijing, China. The patients were approved by their parents and Beijing Children Hospital Research Human Ethics Committee, Beijing, China.

Results

CD4+ and CD8+ T Cells and CD4/CD8 Ratios

Flow cytometric analysis of the T peripheral blood cells revealed a significant change of the balance of CD4+ and CD8+ cells in WS patients compared to controls (Figure 1). The proportion of CD4+ T cells was increased in untreated WS patients in comparison to healthy control infants and topiramate-treated WS patients. The proportion of CD8+
cells was not significantly different between untreated WS patients, treated WS patients and healthy control infants, respectively. No significant changes of the CD4/CD8 ratios were observed between the control group (1.56±0.1) and the topiramate-treated group (1.64±0.38), but the CD4/CD8 ratio was increased in untreated patients (2.25±0.37) in comparison to control infants (p=0.0162) and to treated WS patients (p=0.7979) (Figure 1).

**B Cells and NK Cells**

The proportion of B cells was decreased in topiramate-treated WS patients in comparison to healthy control infants and to untreated WS patients (Figure 2). The proportion of NK cells was decreased in untreated WS patients in comparison to topiramate-treated WS patients and to healthy control infants (Figure 2). There was no significant difference of NK cells between treated WS patients and controls.

**Serum Levels of IgG, IgM, and IgA**

Levels of IgG in serum were decreased in untreated WS patients in comparison to topiramate-treated WS patients and to healthy control infants. Differences between topiramate-treated WS patients and controls were not significant (Figure 3). Serum levels of IgM were slightly increased in untreated WS patients, but further increased in topiramate-treated WS patients in comparison to healthy control infants. However, the differences were again not significant. Serum levels of IgA were decreased in untreated (p<0.05) in comparison to healthy control infants. Levels of IgA in topiramate-treated WS patients were more decreased than untreated group, in comparison to healthy control infants (p<0.01). But the difference between untreated and topiramate-treated WS patients was not significant (Figure 3).

**Discussion**

WS is relatively resistant to treatment as compared to other types of childhood seizures. It is accompanied by impaired cognitive and psychosocial functions. Topiramate is a new antiepileptic drug, which appears to have a broad range of anti-epileptic activities in humans. Clinical trials have shown that topiramate is effective when used in WS. The clinical effects observed in this study are consistent with previous observations showing that topiramate effectively suppresses spasms in WS patients, whereby 44 cases (81.4%) of WS responded to topiramate treatment and 31 cases (57.4%) were seizure-free for more than 6 months. Furthermore, there are evidences to believe that autoimmune mechanisms play a causative role in WS.
is, therefore, of interest to find out, whether immune parameters are also influenced by the new drug in WS patients. In view of the presumptive association of an activated immune system with epilepsy in WS, we sought to obtain information about the humoral and cellular immunological status of WS patients with and without topiramate treatment. In our study the untreated WS patients had increased proportions of CD4+ T cells and increased CD4/CD8 (T-helper/T-suppressor) ratios. This result suggests that a stimulation of the cellular immunity may be connected with WS. The topiramate therapy for WS resulted in normalization of the CD4/CD8 T lymphocyte ratio probably due to an inhibition of the CD4+ T cell population. The CD4/CD8 ratio may, therefore, serve as an indicator of the therapeutic efficacy of topiramate in WS patients. Administration of the drug resulted furthermore in a significant increase of the proportion of NK cells, which were reduced in untreated WS patients compared to controls. NK cells without obvious lytic activity have been found in the target organs of several autoimmune disease11 suggesting that they may participate in the immune regulation against autoantigens. Closer insight into their functional relevance in WS depends on the application of functional assay systems of NK cells in a future study. Serum levels of IgG were lower in untreated WS patients than in healthy control infants, but higher in topiramate-treated WS patients. This finding is compatible with the assumption that the drug restores the normal IgG level in seizure-free infants. Serum levels of IgA were decreased in untreated WS patients in comparison to healthy control children indicating a IgA deficiency in WS patients. This result supports the hypothesis that IgA deficiency is a pathogenetic factor in this type of epilepsy. Topiramate influenced the serum levels of IgA in WS patients similar to other anti-epileptic drugs,12,13 i.e. by further decreasing its serum concentration. Serum levels of IgM did not differ significantly between untreated and topiramate-treated WS patients and healthy control children arguing against a major role of IgM in WS.

In summary, the majority of data obtained in our study demonstrate that topiramate may interfere with the modulated immune system of WS patients mainly in the direction of a normalization.

References