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# Effect of anticonvulsant drugs on interleukins-1, -2 and -6 and monocyte chemoattractant protein-1

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**Abstract** In order to evaluate whether treatment with valproic acid or carbamazepine can modify interleukins and monocyte chemoattractant protein-1, we studied 40 epileptic children and adolescents. We evaluated the patients before and after 1 year of therapy. At the end of follow-up, the patients showed a significant increase of the production of interleukin-1α, interleukin-1β, interleukin-6, and monocyte chemoattractant protein-1; interleukin-2 production was significantly higher only in patients receiving carbamazepine. In conclusion, antiepileptic drugs can influence the immune system by modifying interleukin and chemokine concentrations; these changes seem to be independent of the serum concentrations of these drugs.

**Keywords**: Interleukins • Chemokine • Valproate • Carbamazepine • Epilepsy

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## Introduction

In the last few years, many authors [1–3] have demonstrated that long-term treament with antiepileptic drugs (AEDs) can affect the immune system. More recently, several inflammatory cytokines, including interleukins, have been studied; it has been demonstrated that some cytokines may stimulate adhesion molecules that mediate attraction and adhesion of immune cells to the endothelium [4]; among these adhesion molecules, monocyte chemoattractant protein-1 (MCP-1) is a member of the beta-chemokine subfamily that act in concert with endothelial cell adhesion molecules to attract monocytes and macrophages to sites of inflammation.

The data in the literature about the changes in interleukin levels induced by AEDs are very few and conflicting [3, 5, 6], and there is no study of interleukin and MCP-1 concentrations in patients before and after AED monotherapy.

The aims of our study were to evaluate whether there are differences in interleukin production and MCP-1 in children receiving different AEDs (monotherapy) and to determine any possible relationship between concentrations of these molecules and AED dosage and their serum concentrations.

## Patients and methods

We studied prospectively 40 children and adolescents (19 females, 21 males), aged from 8.9 to 16.2 years, suffering from various types of epilepsy who received different AEDs (monotherapy); the patients were subdivided into two groups according to their therapy: group A, 18 patients (9 females, 9 males) treated with valproic acid (VPA) and group B, 22 subjects (10 females, 12 males) treated with carbamazepine (CBZ). No patient of either group received drugs other than VPA or CBZ during the study.

Patients were recruited from the Department of Pediatrics, University of Chieti; the diagnosis of epilepsy was made after adequate personal history, clinical examination, electroencephalography (EEG), and neuroradiological evaluation. Exclusion criteria for

study participation were seizures due to infections of the central nervous system, seizures due to metabolic disturbances, abnormal neurological examination, and delayed psychomotor development. Moreover, no patients studied had progressive disease of the central nervous system or associated psychiatric disorders. All children showed normal cerebral computed tomography and/or magnetic resonance imaging.

In order to exclude the presence of an immune dysregulation, all patients were studied before AED therapy. None had been treated with adrenocorticotropic hormone (ACTH) or immunological drugs for at least 6 months prior to the study. All children had a normal number of leukocytes per millimeter of blood, normal serum immunoglobulin concentration, and serum C3 and C4 concentrations.

In the two groups of patients, VPA and CBZ were prescribed at the normal dosage, and serum concentrations of AEDs were within the therapeutic range during the study: 71.4±13.9 μg/ml and 7.0±1.9 μg/ml, respectively. No patient had serum concentrations of VPA and CBZ above the therapeutic range. AEDs were administered in two daily doses.

Forty-eight healthy sex- and age-matched children served as controls. Neither patients nor controls had ever suffered from immunological diseases and were free from recent infections. Informed consent was obtained from the parents and the adolescents; consent was obtained also in the control group. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Chieti, Italy.

Before the start of therapy (the first evaluation), we evaluated the concentrations of interleukin-1α (IL-1α), IL-1β, IL-2, and IL-6 secreted by peripheral blood mononuclear cells (PBMC). We also measured plasma concentrations of MCP-1 in the patients.

After 1 year of treatment, we re-evaluated all these parameters in patients and controls; no patient or control had any seizure in the 4 months before the second evaluation.

PBMC were obtained by centrifuging whole blood on a Ficoll-Hypaque density gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). PBMC ($1 \times 10^7$ cells/ml) were cultured on 24-well tissue culture plates and stimulated with phytohemagglutinin (PHA) for interleukin induction and lymphocyte blastogenesis assay. For quantitative measurement of IL-1α, IL-1β, IL-2, and IL-6 in supernatants of PBMC cultures, four specific radioimmunoassay were used (Advanced Magnetic, Cambridge, Mass., USA). The assessments were performed as previously described [6].

Blood samples were collected from each subject and drawn into pyrogen-free test tubes containing EDTA (2.7 mM) and centrifuged at 2,000 rpm at room temperature for 15 min. Plasma samples were collected and stored at -80°C; concentrations of plasma MCP-1 were determined by an enzyme-linked immunosorbent assay (Endogen, Woburn, Mass., USA) and results were expressed in picograms per milliliter.

VPA and CBZ serum concentrations were determined by a capillary gas chromatography method.

### Statistical analysis

Data are presented as mean ±SD. Analyses were performed with the SPSS statistical package (SPSS, Chicago, Ill., USA). The comparisons of groups were performed by ANOVA. Moreover, all variables were included in a multiple regression analysis using the interleukin and MCP-1 values as the dependent variable. $P<0.05$ values were considered significant.

## Results

### Baseline evaluation

At the beginning of the study, there were no significant differences in IL-1α, IL-1β, IL-2, and IL-6 production in response to PHA stimulation between the control group and the two groups of epileptic children. The MCP-1 values were also similar in controls and in the two groups of patients.

### After 1 year of treatment

Group A patients showed a significant increase of the IL-1α, IL-1β, and IL-6 values compared with baseline data and control values (Table 1); in contrast, IL-2 production remained similar to that observed in controls (Table1).

Group B patients showed the same changes in IL-1α, IL-1β, and IL-6 values; these children also had a significant increase in IL-2 production compared with baseline and con-

**Table 1** Interleukin (IL) and monocyte chemoattractant protein-1 (MCP-1) levels of the two groups of patients and controls

| | Group A | | Group B | | Controls | |
|---|---|---|---|---|---|---|
| | Baseline | After 1 year | Baseline | After 1 year | Baseline | After 1 year |
| Sex (M/F) | 9/9 | 9/9 | 12/10 | 12/10 | 26/22 | 26/22 |
| Age (years) | 12.8±3.9 | 13.8±3.9 | 13.4±2.8 | 14.4±2.8 | 13.8±1.9 | 14.8±1.9 |
| IL-1α (ng/ml) | 1.21±0.50 | 3.65±0.54* | 1.08±0.29 | 3.48±0.48* | 1.02±0.32 | 1.10±0.28 |
| IL-1β (ng/ml) | 4.22±1.66 | 9.75±2.03* | 4.41±1.58 | 10.55±2.16* | 4.30±1.21 | 4.36±1.26 |
| Il-6 (ng/ml) | 5.63±2.42 | 12.6±3.48* | 5.45±2.41 | 13.8±3.82* | 5.48±2.43 | 5.51±2.75 |
| IL-2 (ng/ml) | 0.44±0.07 | 0.46±0.06 | 0.49±0.07 | 1.01±0.11* | 0.42±0.05 | 0.43±0.06 |
| MCP-1 (pg/ml) | 64.42±6.93 | 88.46±8.14* | 67.78±6.45 | 90.56±7.98* | 66.76±4.88 | 67.44±5.01 |

* $P<0.001$ vs. baseline and control values

trol values (Table 1). In both groups of patients, MCP-1 was significantly higher than baseline and control evaluations (Table 1).

No correlation was found between the values of interleukins and MCP-1 and AED dosage and serum concentration of the two AEDs in the two groups of patients.

## Discussion

The present study demonstrates that epileptic patients taking AEDs can show changes in interleukin concentrations; in particular, they had increased concentrations of IL-1, both the cell-associated form (IL-1α) and the readily secreted form (IL-1β). This increase was similar in patients receiving VPA and in patients receiving CBZ. In contrast, IL-2 production was significantly increased only in patients receiving CBZ. This is in agreement with previous reports by our [1] and other groups [2] who demonstrated that natural killer cell activity, modulated by IL-2, is increased in patients receiving this AED. Moreover, we found a significant increase in IL-6 production in children treated with CBZ and in those treated with VPA. These changes in interluekin concentrations are in agreement with a cross-sectional study [6] carried out in adult epileptic patients, although this study lacked a pretreatment evaluation of the patients. In contrast, other authors [3] have found that VPA inhibits lipopolysaccharide-induced production of tumor necrosis factor-α (TNF-α) and IL-6 by THP-1 cells, while other authors [5] did not find any significant changes in interleukin concentrations.

IL-6 is a pro-inflammatory cytokine that shares a number of its characteristics with IL-1 [7]; in addition to immune cells, IL-6 is produced by vascular endothelial cells, and is implicated in the recruitment of T cells to the site of inflammation [8].

For the first time, we evaluated MCP-1 concentrations and found a significant increase in both groups of patients compared with baseline evaluation and controls. MCP-1 is the prototype of the C-C chemokine beta subfamily and exhibits its most-potent chemotactic activity toward monocytes and T lymphocytes [9]. In addition to promoting the transmigration of circulating monocytes into tissues, MCP-1 exerts various effects on monocytes, including the induction of superoxide anions and cytokine production [4].

A casual relationship between treatment with these AEDs and these changes of the immune system seems to be established, although the pathogenetic mechanism(s) is not clear. However, it is important to remember that in the last few years cytokines and their receptors have also been found in the central nervous system [10] and both neurons and glia have been shown to produce different interleukins [10–12]. Moreover, it has been demonstrated that convulsant drugs can increase mRNA levels of IL-1β, IL-6, and TNF-α, as well as mRNA of type 2 IL-1 receptor and IL-1 receptor antagonist in rat forebrain within hours of seizure induction [13–15]. More recently, Vezzani et al. [16, 17] demonstrated that IL-1β and its endogenous receptor antagonist (IL-1 Ra) are rapidly induced by seizures in the rodent hippocampus and that exogenous application of IL-1β prolongs kainate-induced hippocampal EEG seizures, probably by enhancing glutamatergic neurotransmission. Therefore, it is possible that one or more interleukins (probably IL-1) may be an epileptogenic cytokine, because its inhibition reduces seizures in animals.

The data of this report are consistent with other studies [1–3, 6] that have demonstrated important immunological changes in epileptic patients treated with different AEDs. Our study provides, for the first time, evidence that some interleukins and chemokines can be modulated by VPA and CBZ. Cytokines may be important for neuronal survival and, recently, some authors [18] have found elevated concentrations of IL-6 in cerebrospinal fluid from patients with recent epileptic seizures, suggesting that cytokine production may be related to epileptic seizures. However, baseline evaluation (before the start of antiepileptic therapy) allows us to exclude the possibility that the immunological abnormalities found in our patients were the result of genetic make up or the convulsive disorder itself; moreover, all children who participated in our study had good control of seizures and did not have any seizures in the 4 months before the second evaluation.

In conclusion, although further and larger studies are needed to evaluate the long-term influence of these AEDs on epileptic patients, our data suggest that CBZ and VPA influence the immune system by modifying interleukin and chemokine concentrations; these changes seem to be independent of the serum concentrations of these drugs.

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