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Short communication

Chronic valproate or levetiracetam treatment does not influence cytokine levels in humans

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ABSTRACT

Purpose: There is growing evidence that complex interactions between seizures and the immune system shape the course of epilepsy. However, systematic analyses of the effects of antiepileptic drugs (AED) on the immune system in humans are rare. We performed a prospective study on the influence of the widely used AED valproate and levetiracetam on interictal immunological parameters.

Methods: 36 patients were prospectively included. 15 were started on valproate (5 female (33%), age 54 ± 27 years, 12 (80%) on monotherapy), 21 on levetiracetam (10 female (48%), age 45 ± 19 years, 17 (81%) on monotherapy). Before treatment and after 3 months, we performed a differential blood count and analyzed the distribution of $CD3^+CD4^+$-, $CD3^+CD8^+$- and $CD4^+CD25^+$-leukocyte subsets using flow cytometry. In addition, we determined the concentrations of IL-1β, IL-6, TNF-α and MCP-1 in the peripheral blood using ELISAs.

Results: Valproate intake resulted in a significant decrease of the total white blood count (6.96 ± 1.23/nl vs. 6.13 ± 1.57/nl, $p = 0.026$) and of absolute count and percentage of neutrophils (4.60 ± 1.05/nl vs. 3.69 ± 1.30/nl, $p = 0.01$; 65.4 ± 7.9% vs. 59.5 ± 11.5%, $p = 0.01$, respectively). The percentage of $CD3^+CD4^+$-lymphocytes dropped significantly (50.4 ± 10.9% vs. 45.3 ± 12.3%, $p = 0.002$). Levetiracetam treatment resulted in a decrease of the percentage of $CD4^+CD25^+$-lymphocytes (26.1 ± 8.0% vs. 21.5 ± 9.2%, $p = 0.01$) but did not significantly alter absolute counts. Neither valproate nor levetiracetam were associated with significant changes in cytokines.

Conclusion: Valproate intake results in profound changes of white blood cell count and subset distribution. Cytokine levels were not influenced by valproate or levetiracetam.

1. Introduction

There is growing evidence that epileptic seizures result in changes of cellular und humoral systemic immunological parameters. Vice versa, immunological processes influence the pathogenesis and course of epilepsies.[1,2]

In previous studies, we found increased counts of leukocytes, neutrophil granulocytes, lymphocytes and NK-cells immediately after seizures in the peripheral blood of patients with temporal lobe epilepsy. $CD4^+$ T-lymphocytes dropped which resulted in a decreased CD4/CD8-ratio.[3] IL-6 serum levels increased immediately postictal and remained elevated for 24 h.[4] Interictal alterations included reduced percentages of B-lymphocytes and $CD4^+$ T-lymphocytes as well as increased percentages of monocytes.[5]

The evaluation of interactions between the central nervous system and immunological processes in epilepsy patients is complicated by the influence of antiepileptic drugs (AED) on the immune system.[1] Market authorization studies of levetiracetam (LEV) showed increased numbers of upper-respiratory tract infections.[6] Valproate (VPA) has various hematologic side effects.[7] However, prospective analyses of the effects of AED on the immune system are rare. Therefore, we analyzed the influence of VPA and

Abbreviations: AED, antiepileptic drug; VPA, valproate; LEV, levetiracetam; CD, cluster of differentiation; IL, interleukin; TNF, tumor necrosis factor; MCP, monocyte chemoattractant protein; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence activated cell scanning; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; APC, allophycocyanin; PerCP, peridinin chlorophyll; PE, phycoerythrin; FSC, forward scatter; SSC, side scatter; CCL 2, chemokine (C–C motif) ligand 2; SD, standard deviation.

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LEV on interictal immunological parameters in patients with active epilepsy.

2. Material and methods

2.1. Patients

Between May 2009 and June 2011, we prospectively included 36 patients of our tertiary epilepsy center who were started on antiepileptic therapy with VPA or LEV. Inclusion criteria were diagnosis of focal or generalized epilepsy, indication for treatment with VPA or LEV, continuation of AED intake for at least 6 months and age above 15 years. All patients provided written informed consent. The study was approved by the local ethics committee. Exclusion criteria were malignant brain tumors, acute infections, other severe neurological or neuroimmunological diseases, interferon or immunoglobulin therapy within the last 6 months, surgery or significant trauma within the last 2 weeks, severe hepatic or renal insufficiency, severe psychiatric disorders and pregnancy. No other medication with known relevant immunological effects was started within the study-period.

2.2. Methods and materials

We analyzed leukocytes, neutrophils, lymphocytes and lymphocyte subset distribution of $CD4^+$ T-lymphocytes ($CD3^+CD4^+$), $CD8^+$ T-lymphocytes ($CD3^+CD8^+$) as well as regulatory T-lymphocytes ($CD4^+CD25^+$) and levels of IL-1β, IL-6, TNF-α and MCP-1 in the peripheral blood of the patients.

Before first intake of the respective drug, 5 ml EDTA blood for differential blood count and analysis of lymphocyte subset distribution and 10 ml serum for cytokine analyses via ELISA were taken. EDTA blood was processed immediately or stored at 4 °C until processing on the same day. Serum tubes were centrifuged immediately at 855 × *g* and 4 °C for 15 min and the serum obtained was stored at −80 °C until further processing.

Differential blood counts were obtained via the central laboratory of our clinic using standardized methods. Lymphocyte subsets were analyzed using 4-color flow-cytometry (fluorescence activated cell scanning (FACS)) as described elsewhere.[5] Briefly, 100 μl EDTA blood were mixed 1:1 with phosphate-buffered saline (PBS) 10% and added to a 96-well microtiter plate. The plates were centrifuged at 300 × *g* for 4 min and the supernatant was discarded. For staining 60 μl of CD3-FITC (Beckman Coulter, Immunotech, France), CD4-APC (Biosciences Pharmingen, USA), CD8-PerCP (BD Biosciences, USA) and CD25-PE (BD Biosciences, USA) were mixed with 80 μl PBS 10%. 5 μl were added to each well and the plate was incubated for 30 min on ice in the dark. Subsequently, red blood cells were lysed with ammonium chloride (10%). After washing, both steps were repeated and the remaining cell pellets were resuspended in 130 μl PBS with 10% fetal calf serum. Lymphocyte subset analysis was performed using a FACSCalibur® and the corresponding CellQuest® Pro software (Becton Dickinson). Lymphocytes were analyzed in a lymphocyte gate resulting from forward scatter- and side scatter-properties. Relative proportions of leukocyte subsets related to all cells in the lymphogate and additionally absolute concentrations of the respective cells were calculated.

Levels of IL-1β, IL-6, TNF-α and MCP-1 were measured using commercially available ELISA kits (Human IL-1 beta ELISA Ready-SET-Go!® and Human CCL2 (MCP-1) ELISA Ready-SET-Go!®, eBioscience, Germany; PeliKine compact™ human IL-6 ELISA kit and PeliKine compact™ human TNFα ELISA kit, Sanquin Reagents, The Netherlands) following manufacturer's instructions. Sensitivity levels were 4 pg/ml for IL-1β, 7 pg/ml for MCP-1, 0.2–0.4 pg/ml for IL-6 and 1–3 pg/ml for TNF-α. If cytokine concentrations were below detection thresholds, the missing value was set to the detection threshold (0.2 pg/ml for IL-1β, 7.8 pg/ml for MCP-1, 0.6 pg/ml for IL-6 and 1.4 pg/ml for TNF-α) to avoid overestimation of differences between baseline and follow-up values.

After being started on VPA or LEV, we scheduled a follow-up visit 3 months later and repeated all measurements as described above.

2.3. Statistics

Categorial variables are given as numbers and percentages, continuous variables as mean ± standard deviation (SD) and median. Intra-individual comparison of parameters obtained before first drug intake and after 3 months was performed. Because Gaussian distribution could not be presumed, the non-parametrical Wilcoxon matched pairs test was used. Statistical analysis was performed using *BiAS für Windows*™ (Hanns Ackermann, University of Frankfurt, Germany). *P*-values < 0.05 were considered statistically significant. Since this study was exploratory, no adjustment for multiple testing was applied.[8]

3. Results

36 patients were included. 21 were started on LEV, 15 on VPA.

3.1. Valproate

Within the VPA group (10 male (67%), age 54 ± 27 years (range 15–87)), 12 patients (80%) were treated with VPA in monotherapy. One patient (7%) was additionally treated with lamotrigine and topiramate, another patient (7%) with carbamazepine and a third (7%) with gabapentine. 7 patients (47%) suffered from generalized, 7 (47%) from focal epilepsies. In one patient (7%), the epilepsy syndrome was unclassified. 13 patients (87%) were free of seizures after 3 months of VPA therapy. The VPA dosage was at this point 903 ± 307 mg/day (median 900 mg/day).

3.1.1. Leukocytes and cytokines

Due to a technical defect, lymphocyte subset distribution could not be obtained in two patients at 3 months ($n = 13$). $CD4^+CD25^+$-levels could not be obtained in another patient ($n = 12$).

Follow-up values at 3 months showed a significant decrease of the total white blood count from 6.96 ± 1.23/nl to 6.13 ± 1.57/nl ($p = 0.026$). Absolute counts of neutrophils decreased from 4.60 ± 1.05/nl to 3.69 ± 1.30/nl ($p = 0.01$) and the corresponding percentages from 65.4 ± 7.9% to 59.5 ± 11.5% ($p = 0.01$).

FACS analysis showed significantly lower percentages of $CD3^+CD4^+$-lymphocytes (50.4 ± 10.9% vs. 45.3 ± 12.3%, $p = 0.002$). Comparison of cytokine levels did not result in any relevant differences (Table 1).

3.2. Levetiracetam

11 (52%) of the 21 patients were male (mean age 45 ± 19 years, range 17–84). 17 patients (81%) received LEV in monotherapy. One patient (5%) additionally received oxcarbazepine and lamotrigine, another (5%) carbamazepine and two (10%) lamotrigine. 17 patients (81%) suffered from focal, 3 (14%) from generalized epilepsy. In one patient the epilepsy was unclassified. 10 of 21 patients (48%) were seizure-free after 3 months. The LEV dosage was at this point 1464 ± 405 mg/day (median 1500 mg/day).

3.2.1. Leukocytes and cytokines

There were no significant changes in leukocytes, neutrophils, total lymphocytes and cytokine levels but the percentage of