Pharmacological Reports 66 (2014) 972–975

Contents lists available at ScienceDirect

Pharmacological Reports

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

Short communication

# Concentration of Il-1β, Il-2, Il-6, TNFα in the blood serum in children with generalized epilepsy treated by valproate

Barbara Steinborn [a], Marcin Żarowski [a,*], Anna Winczewska-Wiktor [a], Marlena Wójcicka [b], Justyna Młodzikowska-Albrecht [a], Jacek Losy [b,c]

[a] Department of Developmental Neurology, Poznan University of Medical Sciences, Poznań, Poland
[b] Department of Clinical Neuroimmunology, Chair of Neurology, Poznan University of Medical Sciences, Poznań, Poland
[c] Neuroimmunological Unit, Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Poznań, Poland

ARTICLE INFO

*Article history:*
Received 14 April 2014
Received in revised form 5 June 2014
Accepted 5 June 2014
Available online 21 June 2014

*Keywords:*
VPA
Generalized epilepsy
Cytokine
Children
IL-6

ABSTRACT

*Background:* The aim of the study was the comparison of concentrations of IL-1β, IL-2, IL-6 and TNFα before and after valproate (VPA) treatment in blood serum in patients with generalized seizures diagnosed and treated in the Department of Developmental Neurology, Poznan University of Medical Sciences from January 2006 to May 2007.

*Methods:* The analysis was conducted in a group of 21 patients with well controlled, generalized seizures (mean age 7.7 ± 4.7 years) before and after 4–6 months of VPA therapy. Quantitative determination IL-1β, IL-2, IL-6 and TNFα were performed with method of enzyme-linked immunosorbent assay (ELISA). The serum drug concentration was determined with the use of fluorescence-polarization-immunoassay system (FPIA).

*Results:* The concentration of IL-6 in blood serum of patients decreased significantly ($p < 0.001$) after 4–6 months of VPA therapy, but concentration of IL-1β ($p = 0.732$), IL-2 ($p = 0.865$), TNFα ($p = 0.079$) did not change significantly. The serum concentration of VPA in all of patients was in therapeutic range (mean 77.53 ± 19.71 μg/ml).

*Conclusions:* The serum level of pro-inflammatory IL-6 in patients with generalized epilepsy decreased in statistically significant way during VPA therapy, so the anti-inflammatory properties of VPA are also important for the effective control of seizure. Due to the incompatibility of reports on the influence of VPA on cytokine system in patients with generalized epilepsy, this problem needs more investigations, especially in the group of children.



## Introduction

Epilepsy is a disorder defined as the occurrence of at least two or more unprovoked seizures, occurrences of which are confirmed by abnormal hypersynchronous discharges of cortical neurons recorded during electroencephalography (EEG) registration [1]. The generalized seizures have an onset recorded simultaneously in both cerebral hemispheres. The lifetime likelihood of receiving a diagnosis of epilepsy is almost 3%, but the prevalence of active epilepsy is about 0.8–1% [1]. The etiology of the most generalized epilepsies is unknown and the seizures are probably genetically determined by disturbances of receptors in central nervous system (CNS) and neurotransmitters or other metabolic problems [1].

In recent years the role of cytokines has been taken into consideration as one of the potential etiologic factors of epileptic seizure. At the beginning of 21st century came out studies concerned with inflammatory reactions, production of proinflammatory cytokines activated in brain after seizures induced in experimental models and in clinical cases of epilepsy [2–5]. The histopathological analysis of brain tissue from patients with epilepsy showed the existence of a chronic inflammation characterized by the neuronal loss, reactive gliosis, malformations of cortical architecture, so the findings confirmed the role of

*Abbreviations:* AED, anti-epileptic drug; CNS, central nervous system; CSF, cerebrospinal fluid; EEG, electroencephalography; ELISA, enzyme linked immunosorbent assay; FPIA, fluorescence polarization immunoassay system; GABA, gamma-aminobutyric acid; IL, interleukin; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF, tumor necrosis factor; VPA, valproic acid, valproate.

* Corresponding author.
*E-mail address:* zarowski@ump.edu.pl (M. Żarowski).

http://dx.doi.org/10.1016/j.pharep.2014.06.005
1734-1140/

inflammation in epileptogenesis [3,5–8]. Glia cells, neurons and lymphocytes T, which are present in CNS, are able to produce and secrete cytokines [9] but they also get through the blood–brain barrier thanks to the passive and active transport (tumor necrosis factor α (TNFα), interleukin-6 (IL-6)) [9]. Proinflammatory cytokines as IL-1β, IL-2, IL-6, TNFα can induce seizures through modulation of glutamatergic transmission [2,4,5,7,8,10], influence the neuronal viability, glial activation and proliferation, survival of cells after injury [5,8], enhance the blood–brain barrier permeability but can also inhibit neurogenesis [4,5,8]. Proinflammatory cytokines i.e. IL-1β, IL-2, IL-6, TNFα are present in healthy brain tissue at very low levels, which increase after ischemic, traumatic and excitotoxic damage, and also after generalized seizures [3,5,8,10]. The experimental evidence based on animals led to conclusion that seizures per se increase the level of cytokines mentioned above in cerebro-spinal fluid (CSF) and blood serum in epileptic patients [4,8]. Valproic acid (VPA) is the first choice antiepileptic drug because it is effective in wide spectrum of seizure types. Some researches indicated that VPA modulates the immune system, however, the mechanism of action is not clear [11]. All functions of VPA in human brain are still unknown but it is known that VPA is gamma-aminobutyric acid (GABA) transaminase inhibitor, it blocks the voltage-gated sodium channels and T-type calcium channels. Moreover, VPA is an inhibitor of the enzyme histone deacetylase 1, hence it is a histone deacetylase inhibitor [12]. There are some studies in the literature concerning the impact of VPA therapy for proinflammatory cytokines concentrations in the blood serum in epileptic patients [7,13]. The doses of VPA must be in therapeutic range in the blood serum to suppress the paroxysmal activity in CNS [1,7,13].

The purpose of the study was the comparison of concentrations of IL-1β, IL-2, IL-6, TNFα before and after VPA therapy, in blood serum in patients with episodes of generalized seizures.

## Material and methods

The analysis was conducted on a group of 21 patients (8 girls and 13 boys) with idiopathic, generalized epilepsy at the age of $7.7 \pm 4.7$ years.

The level of cytokines was assayed before and after 4–6 months of VPA therapy in new diagnosed patients with tonic–clonic seizures who were admitted to the Department of Developmental Neurology, Poznan University of Medical Sciences from January 2006 to May 2007. The study protocol was approved by the bioethics committee.

Children with chronic diseases which could influence the cytokines system i.e. hematological diseases, chronic inflammatory processes, neoplastic diseases and treated were excluded from study. We also excluded patients who needed anti-epileptic drug (AED) polytherapy. The 4–6 month period of treatment was needed to achieve stabile therapeutic dosage and to assess the seizure frequency reduction. Although seizure frequency is the end point used to assess the efficacy of AEDs in clinical studies, but no consensus exists what value (percentage) of seizure frequency reduction is required. In this study we use a 50% seizure-frequency reduction criterion as a study end point.

The blood samples were examined in both stages. The first sample of blood was taken in the morning hours during routinely executed diagnostic investigations inspecting haemopoietic system, liver and kidneys functioning parameters and water and electrolyte balance recommended in patients before the beginning of VPA therapy. The second sample of blood was taken in the morning hours (before morning dose of VPA) to assay VPA serum level before the application of morning dose of drugs in children and adolescents after 4–6 months of VPA therapy. During the examination children had not any signs of infection.

Quantitative determination of cytokines: IL-1β, IL-2, IL-6, TNFα were performed with enzyme-linked immunosorbent assay (ELISA) method (R&D Systems, USA). Human Quantikine ELISA kits for IL-1β, IL-2, IL-6, TNFα estimation were used. Sample concentrations were read from a calibration curve.

The VPA concentration was determined with the use of fluorescence-polarization-immunoassay system (FPIA) system by automatic analyzer TDX, Abbott Diagnostic Division. Quantitative analysis of examined VPA was conducted by the method of calibrating curve indicating dependence between the level of polarization and drug concentration in calibrating solutions.

The nonparametric Shapiro–Wilk *W* test was used to estimate the normality of analyzed data. The Wilcoxon, matched-pairs signed-ranks test was used to compare differences between concentration of IL-1β, IL-2, IL-6, TNFα in the blood serum before and after 4–6 months of VPA therapy.

## Results

The blood serum concentration of IL-6 in epileptic patients decreased after 4–6 months of VPA therapy significantly ($p < 0.001$). Fig. 1C shows the levels of IL-6 in the serum before and during VPA therapy. Concentration of IL-1β, IL-2, TNFα did not change significantly after VPA therapy. The statistical values were as follows: IL-2 ($p = 0.865$); IL-1β ($p = 0.732$), TNFα ($p = 0.079$). Fig. 1A, B and D shows differences between concentrations of IL-1β, IL-2, TNFα before and during VPA treatment. The decrease of TNFα serum level after 4–6 months of VPA therapy is not significant but there is a tendency to lower TNFα concentration. There were no differences between patients who had 4 and 6 month VPA therapy in seizure frequency and concentrations of IL-1β, IL-2, IL-6, TNFα. The level of VPA in the blood serum in all of patients measured before the morning dose of the cure was in therapeutic range (mean $77.53 \pm 19.71$ μg/ml).

## Discussion

The experimental research conducted on animals confirmed the increasing production and secretion of proinflammatory cytokines as IL-1β, IL-6, TNFα at the hippocampus in short time after tonic–clonic seizures [4,5,8]. It has been confirmed in rat models that the neuronal injury extension of hippocampus depended on intensity of IL-1β, IL-6, TNFα synthesis [13]. IL-1β acts pro-convulsively by stimulation of the glutamatergic transmission, but also it inhibits reuptake of glutaminians through astrocytes in hippocampus [3]. IL-1β inhibits glutamate reuptake and increases glutamate release by astrocytes [6]. The significant increase of the IL-6 concentration in the blood serum is found after the tonic–clonic seizures in animal models [3]. IL-6 and TNFα as IL-1β have stimulating influence on glutamatergic neurotransmission [14]. Ichiyama et al. [15] found that levels of cytokines as IL-1β, IL-6, TNFα are elevated in CSF also in children after prolonged febrile seizures. It is proved that IL-2 modified the activity of dopaminergic neurons and had indirect influence on serotonergic, cholinergic, noradrenergic, glutamatergic neurotransmission [6]. De Sarro et al. [16] observed the proconvulsant role of IL-2 on mice model. Although the researches of Sinha et al. [17] concerning serum and CSF concentration of IL-2 in children with epilepsy did not confirm the mentioned above dependence.

VPA which is a short-chain branched fatty with anti-inflammatory, neuroprotective and axon-remodeling effects is one of the basic antiepileptic drug used to treat generalized epilepsy [13]. The influence of VPA on the serum concentrations of proinflammatory cytokines was described in literature, but the results were not unequivocal [18]. Decrease of IL-1β, IL-2, IL-6, TNFα cytokines concentrations during VPA therapy is caused, inter alia, by slowing down activity of the nuclear factor kappa-light-chain-enhancer of