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Original article

Does valproate therapy in epileptic patients contribute to changing atherosclerosis risk factors? The role of lipids and free fatty acids

Elżbieta Płonka-Półtorak[a,*], Paweł Zagrodzki[b,c], Jadwiga Kryczyk-Kozioł[c], Tuomas Westermarck[d], Pekka Kaipainen[d], Markus Kaski[d], Faik Atroshi[e]

[a] Antiepileptic Outpatient Clinic, Voivodship Clinical Hospital No 2, Rzeszów, Poland
[b] Henryk Niewodniczański Institute of Nuclear Physics, Kraków, Poland
[c] Department of Food Chemistry and Nutrition, Medical College Jagiellonian University, Kraków, Poland
[d] Rinnekoti Research Centre, FIN 02980 Espoo, Finland
[e] Department of Pharmacology and Toxicology, ELTDK, FIN 00014 University of Helsinki, Finland

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ABSTRACT

Background: We aimed to demonstrate the relationship between the valproate (VPA) treatment *versus* lipid and serum free fatty acids (FFAs) profiles to be the potential atherosclerosis risk factor in epileptic patients.
Methods: Fasting blood samples were taken from 21 adult VPA-treated patients and 21 controls. The profiles of lipids, FFAs, clinical parameters and body mass index (BMI) were evaluated.
Results: No significant differences between the study group and controls were found for any of the studied parameters. However, significant differences in the total cholesterol (CHOL), low-density-lipoprotein cholesterol (LDL), triglycerides, the CHOL/HDL (high-density-lipoprotein cholesterol) ratio, and Atherogenic Index of Plasma were observed for overweight patients when compared to those of normal weight. Patients with uncontrolled epilepsy tended to have significantly lower palmitic acid level than seizure-free patients. Oleic acid was found to be positively correlated with VPA concentration for patients with uncontrolled epilepsy, and with the dose corrected VPA concentration for all the patients. The acid was however negatively correlated with stearic acid for both the controls and the patients with uncontrolled epilepsy. PLS method revealed CHOL, LDL, triglycerides and myristic acid to be positively interrelated for the whole group under the study, whereas these parameters were found to be negatively correlated with VPA concentration, and positively with BMI. Furthermore, high sensitivity C-reactive protein was found to be negatively correlated with palmitic acid levels.
Conclusion: Overweight VPA-treated patients are exposed to higher risk of atherosclerosis. Alterations in FFAs are likely to depend on seizures control, and on VPA levels.

Introduction

Although some epilepsy cases are resolvable instantly [1], for the majority of epileptic patients a regular and prolonged treatment with antiepileptic drugs (AEDs) is necessary to reduce the risk and consequences of recurrent seizures. Recently, new evidences have been reported for AEDs therapy to be associated with chronic adverse metabolic effects, including abnormalities in weight gain and modified lipid profile, which are well known risk factors of atherosclerosis [2–4]. Interestingly, the new AED generation despite their better pharmacokinetic properties may have unfavourable impact on circulatory markers for vascular diseases [5].

Valproate (2-*n*-propylpentanoic acid; VPA), a classical AED, is a simple branched fatty acid found highly effective in epilepsy

Abbreviations: AED, santiepileptic drugs; AIP, Atherogenic Index of Plasma (Log_{10}[TG/HDL]); BMI, body mass index; CHOL, total cholesterol; CHOL/HDL, total cholesterol/HDL cholesterol ratio (Castelli's Risk Index I); CRP, C-reactive protein; CVD, cardiovascular disease; Epi time, time since the onset of epilepsy; FFAs, free fatty acids; GABA, γ-aminobutyric acid; HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein cholesterol; PA, palmitic acid; PLS, Partial Least Square method; Seizures/m, number of seizures (focal) over the month proceeding examination; Seizures/y, number of seizures per year; TG, concentration of triglycerides; VPA, valproate; VPA conc., valproate concentration in serum; VPA time, time of VPA monotherapy (years); Z, dose corrected VPA conc.

* Corresponding author.
E-mail address: elapp13@wp.pl (E. Płonka-Półtorak).

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treatment for nearly fifty years. Although the mechanisms of its action have not yet been fully understood, the main effects include VPA influence on brain γ-aminobutyric acid (GABA) metabolism, and interference with T-type calcium channels [6]. Moreover, similarly to free polyunsaturated fatty acids, VPA was postulated to modify biophysical properties of the cellular membrane near the sodium channels [7]. Nevertheless, VPA influence on GABA and the catecholamine system in hypothalamus and pancreas is most likely to be associated with increased appetite and stimulated insulin secretion by pancreatic β-cells [8,9]. On the other hand, hyperinsulinemia and insulin resistance, frequent side effects of valproate treatment, especially in obese patients, may also result in increased appetite and weight gain [10].

VPA is primarily bound to plasma albumins, and with raised plasma drug level the binding extent is reduced, which causes valproate free fraction to grow [11]. It competes with free fatty acids (FFAs) for albumin binding [12] so it enhances long-chain FFAs availability for stimulation of β-cells to increase insulin production [8,13]. Furthermore, the structures of VPA and FFAs are similar, which may be responsible for direct modulation of pancreatic islet cell, as found in *in vitro* study [14]. In the liver, VPA, an enzyme inhibitor, also may interfere with insulin metabolism and lead to a raise in its concentration in the serum and the stimulation of lipogenesis [15]. Both elevated insulin and FFAs cause higher glucose secretion in liver, and enhances production of triglycerides (TG) and very-low-density lipoprotein, giving raise to the risk of type 2 diabetes, obesity, metabolic syndrome, and finally atherosclerosis [13]. VPA induced decrease in serum carnitine, inhibited mitochondrial β-oxidation of long chain fatty acids, which result in increased level of FFAs, provide another plausible mechanism for insulin resistance, body weight gain and atherosclerosis [11,16].

The crucial role of oxidative stress in pathogenesis of atherosclerosis, as well as other related diseases, has been well established. Lipids are susceptible to oxidation since their double bounds are highly reactive. Oxidation of low-density lipoprotein cholesterol (LDL) due to reactive oxygen species causes endothelial dysfunction and leads to foam cells formation. However, high-density lipoprotein cholesterol (HDL) plays a protective role against lipoprotein oxidation mainly by increasing paraxonase activity, preventing inflammation and enhancing cholesterol efflux to the liver [17].

Recently, an important role of oxidative stress in VPA therapy, and in epilepsy itself, has been postulated by which acceleration of atherosclerotic processes is facilitated [2,18].

Under the present study we aimed at evaluating possible interactions between the lipid profile, including free fatty acids, and some other relevant biochemical parameters which though not directly related to the anti-oxidative system, reflect the nutritional and metabolic status in epileptic patients treated with valproate monotherapy. The practical aspect of the study was to identify fatty acids which may influence, positively and negatively, atherosclerosis risk factors, and thus imply the need for appropriate diet modification.

Patients and methods

Fasting blood samples were taken from 21 outpatient epileptic adult patients (aged 19–41y; 11 women and 10 men) receiving VPA in monotherapy, and from healthy gender-matched controls living in the same area. Pregnant and breast-feeding women, patients with progressive brain pathology, and with other chronic treatment were excluded from the study. Detailed demographic and clinical characteristics of the patients and controls were summarized elsewhere [19]. Over the sampling period no restrictions on dietary habits were imposed. Patients filled in a standardized questionnaire on their lifestyle and dietary habits. The local Ethical Committee of the Regional Chamber of Physicians approved the plan of the study.

Blood samples treatment as well as analytical methods to determine biochemical parameters and total VPA serum concentrations (VPA conc.) were also described elsewhere [19]. Serum total cholesterol (CHOL), high-density-lipoprotein cholesterol, triglyceride, free carnitine and high sensitivity C-reactive protein (hs-CRP) levels were measured with an automated spectrophotometric analyzer (Konelab, Model 20× Ti, ThermoFisher Scientific) equipped with standard kits by ThermoFisher Scientific (USA). Low-density-lipoprotein cholesterol was estimated by means of the Friedewald formula. FFAs were extracted from serum according to the method by Itaya and Ui [20]. The serum samples were kept at −20 °C prior to the analysis. FFAs were measured by means of Agilent 1100 HPLC System. The procedure was conducted according to Shimomura et al. [21]. Seronorm Lipid control serum was used as the control, percentage of fatty acids was computed. Atherogenic Index of Plasma (AIP) [22,23], and CHOL/HDL index (Castelli's Risk Index I) [24] were calculated.

Statistical analyses

Comparisons between study and control groups were performed with either a Student *t*-test for parameters with normal distributions and homogenous variances, or a Mann-Whitney test in all other cases. The differences in p values < 0.05 were considered to be significant. The Pearson correlation coefficients (R) were calculated for pairs of parameters. A Fisher two-tailed test was used to assess the significance of the difference between two Pearson correlation coefficients. This part of statistical evaluations was done with STATISTICA PL v.10 package (StatSoft, USA), and by means of the online calculator (http://faculty.vassar.edu/lowry/rdiff.html) for Fisher R-to-z transformation.

A partial least square (PLS) model was used to reveal the correlation structure between the investigated parameters [25]. The set of dependent parameters consisted of diagnostic biochemical parameters useful in assessing arteriosclerosis risk, while the fatty acids profile as well as anthropometric parameters, along with free carnitine and antiepileptic therapy related parameters such as the number of seizures in the a month proceeding examination [Seizures/m], and year [Seizures/y], VPA conc., duration of VPA therapy [VPA time] and epilepsy course [Epi time] were taken as predictors. The parameters with weights >0.3 were assumed to be correlated. The association between two parameters was quantified by calculating their correlation weights, *i.e.* for specific pairs of the considered parameters we calculated the algebraic product of their corresponding weights and the cosine of the corresponding angle, *i.e.* the angle determined by two lines connecting the origin with coordinates of both parameters on the PLS plot. The calculations of PLS model were carried out with the package SIMCA-P v.9 (Umetrics, Sweden). The correlation weights were calculated with software delivered by MP System Co. (Poland).

Results

The differences between the study group and the controls were found to be of no statistical significance for all parameters (Table 1). Similarly, no differences between men and women were revealed, or between the patients from the group with VPA longer treatment (7–14 y) in comparison to the patients to whom VPA was administered over a shorter period. The only difference, the mean daily VPA dose, notably 595.0 ± 195.0 *vs.* 904.5 ± 373.1 mg/d, was found between the younger patients, *i.e.* under 26 years old (26 y – median value for the whole group of patients) and the older