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Valproate Treatment and Platelet Function: The Role of Arachidonate Metabolites

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Summary: *Purpose:* Valproate (VPA) is an extensively used drug in the therapy of epilepsies. One of the most frequently reported side effects of VPA is hemorrhagic diathesis. Some authors emphasized the decreased platelet count as the basis of VPA-induced hemorrhagic diathesis, but some reports suggested that a significant proportion of patients with normal platelet count may still have an altered platelet function. The mechanism of the VPA-induced platelet dysfunction has not yet been elucidated. A determining element of platelet functions is the arachidonate cascade. Present ex vivo experiments were designed to determine whether a relation exists between the incidence of hemostasis caused by VPA and the effect of this drug on the arachidonate cascade of platelets.

Methods: Platelets were isolated from patients receiving long-term VPA treatment (serum level, 36.04 ± 16.12 μg/ml; n = 10) or carbamazepine (CBZ) treatment (serum level, 5.24 ± 2.67 μg/ml; n = 10) and were labeled with [^{14}C]arachidonic acid. (CBZ-treated patients were chosen as a control group, because CBZ causes blood dyscrasias similar to those elicited by VPA, but there has been no report that CBZ induces a platelet dysfunction.) The ^{14}C-eicosanoids were separated by means of overpressure thin-layer chromatography and determined quantitatively by liquid scintillation.

Results: Even when the mean plasma concentration of the drug was low, VPA treatment reduced the activity of the arachidonate cascade in platelets. VPA effectively inhibited the cyclooxygenase pathway and the synthesis of the strong platelet aggregator thromboxane A_2.

Conclusions: Inhibition of the platelet arachidonate cascade may contribute to the platelet-function alterations caused by VPA. **Key Words:** Valproate—Platelets—Thromboxane—Prostaglandin—Hemostasis.

Valproate (VPA) is one of the drugs most extensively used in the therapy of generalized epilepsies. The manufacturers' descriptions of VPA emphasize a risk of rare but potentially severe hepatic toxicity. Another important side effect of VPA administrations is hemorrhagic diathesis. Patients taking VPA have demonstrated alterations in hemostasis, including thrombocytopenia (1), a platelet dysfunction (2), a decreased fibrinogen plasma level (3), and a low von Willebrand factor antigen titer (4), as well as clinically significant bleeding (5,6). Several mechanisms have been proposed to explain these hematologic disturbances, including the formation of a platelet antibody (7), a direct platelet membrane effect (8), and bone marrow suppression (9). Some authors emphasized the decreased platelet count as the basis of VPA therapy–induced hemorrhagic diathesis. On the other hand, Gidal et al. (2) suggested that a significant number of patients treated with VPA who have platelet counts within the normal range may still have an altered platelet function. This is relevant because, for example, clinically significant intraoperative bleeding in a child receiving VPA has been reported despite a normal platelet count (6). The exact mechanism of the VPA-induced platelet dysfunction has not yet been elucidated.

A determining element of the platelet functions is the arachidonate (AA) cascade of the platelets. This is initiated by a receptor-mediated liberation of AA from phospholipids on the action of phospholipases (10). The free AA is metabolized to lipoxygenase products [12-hydroperoxy-5,8,10,14-eicosatetraenoic acid (12-HPETE) and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE)] and also to cyclooxygenase metabolites [thromboxane (Tx) A_2, prostaglandin (PG) D_2, PGE_2, and $PGF_{2\alpha}$]. The formation of TxA_2 is accompanied by the production of 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT), with the concomitant release of malondialdehyde (MDA; 11). Quantitatively and functionally, the most important product of the cyclooxygenase metabolites of the thrombocytes is TxA_2, which is a potent proaggregatory substance, causing platelet shape change, aggregation, and the release of granule contents (12). PGD_2, on the other

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hand, is a potent antagonist that inhibits platelet activation through increased cyclic adenosine monophosphate (cAMP). PGE_2 and $PGF_{2\alpha}$ also affect platelet activation, although to a much lesser extent that TxA_2 and PGD_2 (13). Inhibition of the platelet cyclooxygenase, a beneficial effect of nonsteroidal antiinflammatory drugs (NSAIDs) in anticoagulant therapy, can lead to an increased bleeding tendency. The lipoxygenase products have no direct effects on platelet activation, but they may modulate the AA metabolism via the cyclooxygenase pathway (14).

Our ex vivo experiment was designed to determine whether a relation exists between the incidence of hemostasis disorders caused by VPA and the effect of this drug on the AA cascade of the platelets.

METHODS

Patients

Patients were recruited from the Department of Neurology, Albert Szent-Györgyi Medical University of Szeged, Hungary. Inclusion criteria for study participation included their abstaining from any medication known to alter platelet activation, aggregation, and release reaction. After giving written informed consent, 10 young male patients (mean age, 22.90 ± 7.25 years) participated in the study. All had been on a stable VPA monotherapy regimen (Depakine Chrono: Sanofi Winthrop/Chinoin, Budapest, Hungary; or Convulex: Gerot Pharmazeutika, Wien, Austria) for ⩾120 days before evaluation. The mean VPA dosage was 8.38 ± 2.03 mg/kg/day. The mean total plasma VPA concentration was 36.04 ± 16.12 μg/ml. Seizure types included idiopathic generalized epilepsy (n = 7) and cryptogenic or symptomatic partial epilepsy (n = 3). The control group consisted of 10 male patients (mean age, 31.50 ± 11.41 years) receiving stable carbamazepine (CBZ) monotherapy (Neurotop R: Gerot Pharmazeutika, Wien, Austria; or Tegretol CR: Novartis, Basel, Switzerland), who were subject to the same inclusion criteria. The mean CBZ dosage was 8.10 ± 2.86 mg/kg/day. The mean total plasma CBZ concentration was 5.24 ± 2.67 μg/ml. Seizure types involved cryptogenic or symptomatic partial epilepsy (n = 10). CBZ-treated patients were chosen as control group because CBZ causes blood dyscrasias (leukopenia and thrombocytopenia) similar to those elicited by VPA (15), but there has been no report of CBZ inducing a platelet dysfunction. Investigations were performed with the permission and approval of the Human Investigation Review Board of Albert Szent-Györgyi Medical University, Szeged, Hungary.

Study design

Patients and controls were scheduled to report to the clinical laboratory between 8 a.m. and 10 a.m. after an overnight fast to minimize the impact of fluctuations in plasma free fatty acids on the plasma concentrations of the drugs.

Analytic methods

Cell counting (red blood cells, white blood cells, and platelets) and other hematologic parameters [mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV)] were measured with a Sysmex CC780 instrument (Medical Electronics Ltd., Kobe, Japan). The Ivy bleeding-time test was performed on the forearm. The coagulation time was determined by the Burker method. The plasma fibrinogen level was determined with the Fibri-Prest reagent on an Amelung Coagulometer KC1A (Diagnostica-Stago, Asnieres, France). Serum aspartate aminotransferase (ASAT), alanine aminotranferase (ALAT), alkaline phosphatase (ALP), and γ-glutamyl-transpeptidase (GGT) levels were assayed with a Hitachi 917E clinical chemistry analyzer (Boehringer Mannheim, Mannheim, Germany). Plasma concentrations of VPA and CBZ were determined by fluorescence polarization immunoassay technology on a TDX Analyzer (Abbott Laboratories, Chicago, IL, U.S.A.).

Study of the platelet AA cascade

Platelets were isolated from venous blood samples by differential centrifugation, as described previously (16). From the platelets of each patient, two incubation samples were prepared. Each incubation mixture contained 10^8 cells in 1 ml buffered Medium 199. All samples preincubated at 37°C for 5 min and then incubated with the tracer substrate, L-[^{14}C]AA (0.172 pmol, 3.7 kBq). Ten minutes later, the enzyme reaction was stopped by bringing the pH of the incubation mixture to 3 with formic acid. The ^{14}C-eicosanoids were separated by means of overpressure thin-layer chromatography and determined quantitatively by liquid scintillation, as described previously (16).

Data analysis

Statistical comparisons were made by unpaired *t* test. Associations were measured with the Pearson *r* correlation coefficient.

RESULTS

AA cascade of platelets

The platelets of the VPA-treated patients synthesized less 12-HETE than did those of the CBZ-treated patients. This difference ($p = 0.06$) was close to the significance level. The platelet cyclooxygenase activity in the VPA-treated patients was significantly decreased ($p = 0.014$). The total metabolite formation in the AA cascade of the platelets of the VPA-treated patients was decreased significantly ($p = 0.018$) relative to that of the CBZ-treated patients. The main cyclooxygenase metabolite of the platelets is TxA_2. The synthesis of TxA_2 (the stable metabolite of which, TxB_2, was determined) was significantly decreased ($p = 0.038$) in the platelets of the VPA-treated subjects. The synthesis of TxA_2 is accompanied

by the parallel production of HHT directly from PGH_2 with the release of MDA. The synthesis of HHT was also decreased, and this difference ($p = 0.055$) was close to the significance level. The syntheses of other cyclooxygenase metabolites ($PGF_{2\alpha}$, PGD_2, and PGE_2) were likewise decreased in the platelets of the VPA-treated patients, but only that of PGD_2 was decreased significantly ($p = 0.003$; Table 1).

Other laboratory examinations

The analysis of liver enzymes, ASAT, ALAT, ALP, and GGT, did not reveal significant differences between the VPA-treated and the CBZ-treated patients; with the exception of GGT, the values were in the physiological range. The serum GGT level was significantly higher ($p = 0.004$) in the CBZ-treated patients compared with the VPA-treated patients, and slightly above the upper limit of the normal range. The numbers of the corpuscular elements of the blood (platelets, white blood cells, and red blood cells), the parameters of the red blood cells (MCH, MCHC, and MCV) and the hemostatic parameters (plasma level of fibrinogen, bleeding time, and coagulation time) were in the physiological ranges, and there was no significant difference between the two patient groups (Table 2).

Interpatient correlations

No statistically significant correlations were found between the Tx production of the platelets and the plasma VPA concentrations ($r = 0.402$), the daily dose of VPA ($r = 0.002$), or the duration of VPA treatment ($r = 0.3$).

DISCUSSION

VPA is an effective, widely used AED, but there have been a number of reports that VPA treatment causes alterations in hemostasis, and it is believed by some researchers to increase surgical bleeding (5,6). The most frequently noted cause of these hemostatic abnormalities is thrombocytopenia. Additionally, VPA treatment is known to cause a platelet dysfunction (2). One of the most important elements in the regulation of the platelet functions is the AA cascade of the platelets.

Our data demonstrate that, even when the mean plasma concentration of the drug was low (36.04 ± 16.12 μg/ml), it was less than the usual therapeutic range, but the patients were seizure free according to the clinical symptoms and EEG signs, the applied VPA treatment reduced the activity of the AA cascade in the platelets. VPA inhibited the cyclooxygense pathway more effectively than the lipoxygenase pathway.

The hematologic parameters and the serum liver enzyme levels of the patients were in the physiological range. A higher therapeutic dose of VPA might alter these results.

TxA_2 is functionally the most important product of the platelet AA cascade, and its synthesis was inhibited by VPA. The Tx synthesis of the platelets is accompanied by the parallel production of HHT and MDA (11). Our results are in agreement with the findings of Voss et al. (17), who demonstrated a decreased MDA production in patients receiving VPA therapy.

Different types of platelet-activator substances (collagen, ADP, thrombin, AA, and ristocetin) are used to test the platelet function. Thrombin induces an AA cascade-independent, whereas AA elicits an AA cascade-dependent platelet activation and aggregation (18). Gidal et al. (2) reported a decreased AA-induced platelet activation (the platelet ATP release was measured) and aggregation in VPA-treated subjects, but they did not observe differences in thrombin-induced platelet activation as compared with their control group. The results of Gidal et al. (2) demonstrated that the VPA-induced platelet dysfunctions involve alterations in the AA cascade. We found a lower activity of the AA cascade and a decreased production of TxA_2 in the platelets of patients receiving VPA therapy, which lends support to the findings of Gidal et al. (2).

In contrast with Gidal et al. (2), who reported dose-related effects of VPA on the platelet functions, we did not find correlations between the Tx synthesis of the platelets and the plasma concentration or the applied dose of VPA, which may suggest idiosyncratic action of the drugs, although it is possible that the reason for the lack of an apparent concentration–effect relation could be the low mean VPA plasma concentrations.

The mechanism of action of VPA on the platelet AA cascade has not yet been elucidated. VPA may possibly cause alterations in the membrane phospholipids of the platelets, as in the erythrocytes (8), or reduce the calcium

TABLE 1. *Effects of valproate treatment on the arachidonate cascade of platelets*

	$PGF_{2\alpha}$	PGE_2	PGD_2	HHT	TxB_2	CO	LO	Total
Control	2,715 ± 221	3,347 ± 208	7,864 ± 368	8,723 ± 874	31,763 ± 2,275	54,412 ± 3,283	161,392 ± 4,203	215,804 ± 6,651
VPA	2,218 ± 143	2,929 ± 151	5,624[a] ± 543	6,555 ± 597	24,942[a] ± 2,026	42,268[a] ± 2,970	138,885 ± 10,403	181,154[a] ± 11,559

Each value represents the mean ± standard errors of radioactive arachidonate metabolites in dpm.

Control, group of patients receiving stable carbamazepine monotherapy (n = 10); VPA, group of patients receiving stable valproate monotherapy (n = 10); PG, prostaglandin; HHT, 12-L-hydroxy-5,8,10-heptadecatrienoic acid; TxB_2, thromboxane B_2; CO, cyclooxygenase metabolites; LO, lipoxygenase metabolites; Total, sum of arachidonate metabolites (Total = CO + LO).

[a] $p < 0.05$, compared with the control.

TABLE 2. *Effects of valproate treatment on liver enzymes and hematologic paramters*

	Control	VPA	Normal range
ASAT (U/L)	22.80 ± 3.46	22.90 ± 1.31	<37
ALAT (U/L)	24.00 ± 3.58	21.60 ± 3.47	<40
ALP (U/L)	213.40 ± 22.77	190.30 ± 24.51	<270
GGT (U/L)	54.50 ± 9.27	21.20 ± 4.14[a]	11–50
Fibrinogen (mg/dl)	308.30 ± 34.05	279.30 ± 19.61	200–450
Coagulation time (min)	6.25 ± 0.38	6.01 ± 0.36	5–8
Ivy bleeding time (min)	5.47 ± 0.62	4.86 ± 0.55	3–7
Platelet count ($10^3/\mu l$)	292 ± 29	243 ± 28	150–450
WBC count ($10^3/\mu l$)	5.34 ± 0.59	5.97 ± 0.59	3.4–10
RBC count ($10^6/\mu l$)	4.91 ± 0.07	4.97 ± 0.07	2.4–5.6
MCH (pg/cell)	31.03 ± 0.38	31.42 ± 0.49	26–34
MCHC (g/dl)	31.87 ± 0.49	32.03 ± 0.34	31–36
MCV (fl/cell)	97.90 ± 1.32	98.10 ± 1.41	80–100

The tabulated data are expressed as mean values ± standard errors.

Control, group of patients receiving stable carbamazepine monotherapy (n = 10); VPA, group of patients receiving stable valproate monotherapy (n = 10); Normal range, physiological range of parameters in our laboratory; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyl-transpeptidase; WBC, white blood cells; RBC, red blood cells; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume.

[a] $p < 0.05$, compared with the control.

current in the platelets, as in the neurons (19), modifying the intracellular calcium level, which plays an important role in the activation of the AA cascade of the platelets. However, Kusumi et al. (20) reported that VPA did not alter the calcium concentration in the platelets.

There is a controversy as to whether VPA induces an increased bleeding tendency (5,6,21). Our data revealed that a low dose of VPA inhibits the Tx production of the platelets, which may lead to decreased platelet activation and aggregation. Our results draw attention to the possibility of the administration to patients with VPA therapy of other drugs with inhibitory effects on the platelet function, thereby increasing the risk of bleeding.

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