

CLINICAL AND LABORATORY OBSERVATIONS

Protein C deficiency related to valproic acid therapy: A possible association with childhood stroke

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We report a case of stroke in a child with acquired protein C deficiency receiving valproic acid (VPA). To investigate the possible association of VPA with protein C deficiency, protein C levels were measured in 20 children receiving VPA monotherapy and 20 children receiving other anticonvulsants. Protein C levels were reduced in up to 45% of the VPA-treated subjects.

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Valproic acid has been demonstrated to cause alterations of hemostasis including abnormal bleeding time, qualitative and quantitative platelet abnormalities, decreased fibrinogen levels, and clinically significant bleeding.¹⁻⁷ The effect of VPA on the natural anticoagulants protein C, protein S, and antithrombin has not been reported. An 18-month-old child had a stroke while receiving VPA therapy for a seizure disorder; she was found to have low plasma levels of protein C, which normalized after discontinuation of the anticonvulsant.

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There have been no previous reports of thrombosis or stroke associated with VPA therapy. Therefore a study was undertaken to evaluate the levels of protein C and the other natural anticoagulant proteins and their possible association with VPA therapy in children.

CASE REPORT

The propositus is a 5-year-old girl who developed a seizure disorder at 2 months of age. Birth history was uneventful. An evaluation to determine the cause of the seizures was unrevealing. Head magnetic resonance imaging, performed at the ages of 4 months and 19 months, showed mild prominence of the ventricles, but findings were otherwise normal. Although she initially received phenytoin, she was switched to treatment with VPA after the development of an allergic rash. Development was delayed, but she was otherwise in good health. At 20 months of age, she presented with a seizure and right-sided hemiparesis clinically

consistent with a stroke. A magnetic resonance imaging scan of the brain showed diffuse gyral swelling of the left hemisphere. The child was referred, at 24 months of age, for further evaluation. Physical findings at that time included persistent right-sided hemiparesis. Repeat magnetic resonance imaging of the brain revealed diffuse cortical volume loss on the left side, consistent with a previous diffuse ischemic injury. An evaluation to rule out an underlying prothrombotic disorder was performed at age 24 months. A complete blood count was normal except for a platelet count of $113 \times 10^3/\mu\text{L}$. A serum VPA level was 111 $\mu\text{g}/\text{mL}$. The fibrinogen level was 184 mg/dL. Plasma levels of antithrombin and protein S were normal. Results of tests for a lupus anticoagulant and anticardiolipin antibody were negative. Results of polymerase chain reaction testing for the Arg⁵⁰⁶ to Gln mutation of the factor V gene (factor V Leiden) and the 20210 G to A polymorphism in the 3'-untranslated region of the prothrombin gene were normal. The plasma protein C antigen level was 44%

ELISA Enzyme-linked immunosorbent assay
VPA Valproic acid

(normal for age, $\geq 58\%$), and protein C anticoagulant activity was 30% (normal for age, $\geq 60\%$.) At age 28 months, protein C testing was repeated (antigen level, 50%; anticoagulant activity,

28%). There was no family history of thrombosis, and protein C levels in both parents were normal. VPA was gradually discontinued by 35 months of age, and the child began receiving lamotrigine and carbamazepine. Studies at 39 months of age showed a rise in protein C levels to normal (antigen, 97%; anticoagulant activity, 73%).

METHODS

To determine the possible effects of VPA on the natural anticoagulant proteins, children who were followed up in the Division of Neurology at the Children's Hospital Medical Center and who were receiving VPA as their only anticonvulsant medication were selected (group I) and compared with a similar group of children receiving other anticonvulsant therapy (group II). The study was approved by the Review Board on Investigations Involving Human Beings of the Children's Hospital Medical Center. Appropriate informed consent was obtained for all participants. The age range of children eligible for the study was 6 months to 16 years. After a medical history and an inventory of current medications were taken, blood samples were obtained for the determination of a complete blood count and levels of VPA, alanine aminotransferase, serum albumin, fibrinogen, protein C, protein S, antithrombin, and D-dimer. Prothrombin time, activated partial thromboplastin time, and plasma levels of factor X antigen were measured to rule out possible effects of the anticonvulsants on the vitamin K-dependent coagulation proteins. Normal values of the anticoagulant proteins for age were derived from a previous study in 197 otherwise healthy children aged 6 months through 17 years (inclusive) who were admitted for elective same-day surgery. To define normal levels for age, children were divided into 4 non-overlapping groups: ages 6 months to 1 year, 1 to 5.5 years, 5.5 to

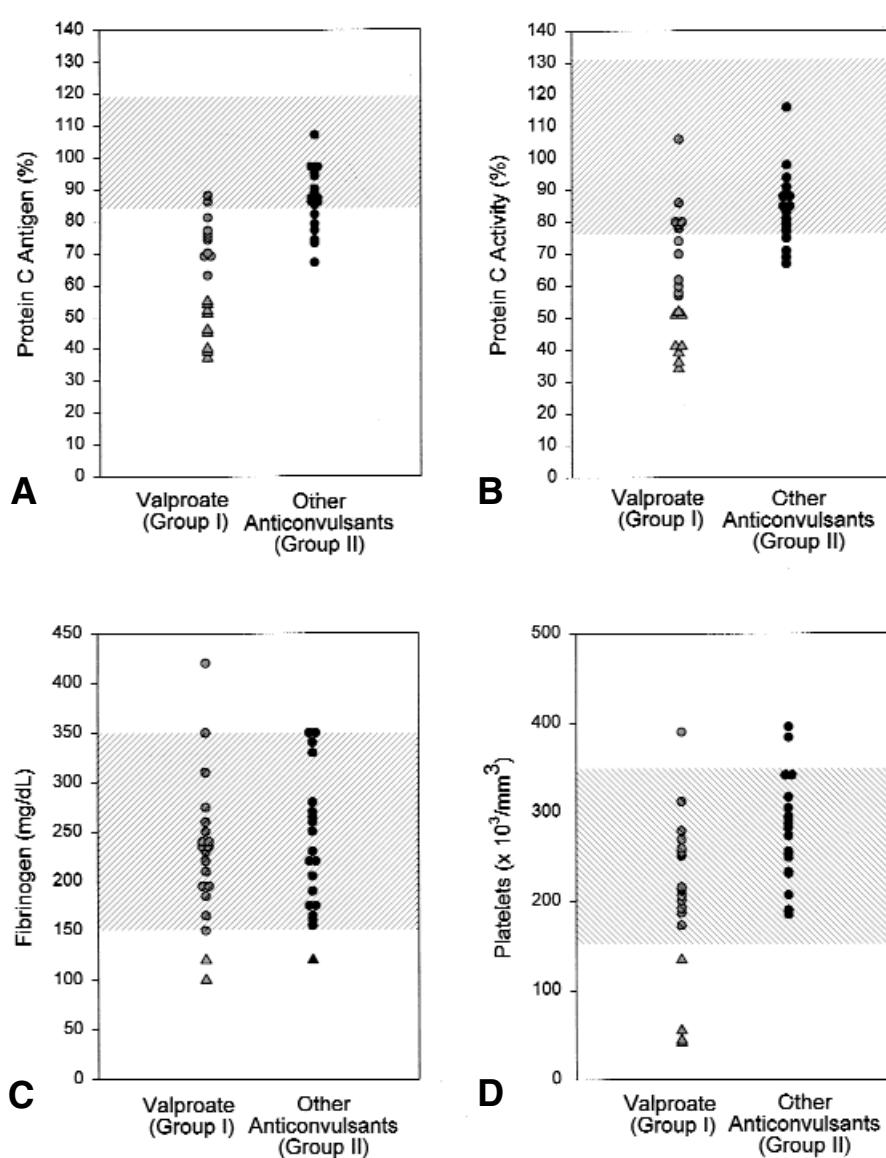


Figure. Scatter graphs showing individual values for plasma concentrations of protein C antigen (A), protein C activity (B), fibrinogen (C), and whole blood platelet count (D) in children treated with VPA (group I) versus children treated with other anticonvulsants (group II). Circles represent normal values for age. Triangles represent abnormal values ($\leq 5^{\text{th}}$ percentile) for age. Normal values for protein C antigen and functional activity were derived from a study of healthy children for each of 4 non-overlapping age groups: 6 months to 1 year; 1 to 5.5 years; 5.5 to 11.2 years; and 11.2 to 16.9 years (see Methods). Shaded area represents normal adult range and is provided for reference only.

11.2 years, and 11.2 to 16.9 years. The cutoff for the lower limit of normal was set at $\leq 5^{\text{th}}$ percentile for each age group. The study of healthy children was approved by the Review Board on Investigations Involving Human Beings, and appropriate informed consent was obtained. Normal values for adults were derived from a study of 40 healthy adults receiving no medication.

Protein C antigen was determined by

enzyme-linked immunosorbent assay with the Aserachrom Protein C kit (Diagnostica Stago, Asnieres-Sur, France). Protein C function was measured as chromogenic activity with the Coamatic Protein C kit (Chromogenix, Mölndal, Sweden). Protein S total and free levels were determined by ELISA with the Aserachrom kits (Diagnostica Stago). Antithrombin level was determined by the Spectrolyse antithrombin

Table. Comparison of coagulation proteins in group I study subjects receiving VPA (n = 20) compared with group II subjects receiving other anticonvulsants (n = 20)

	Group I (n = 20)	Group II (n = 20)
	No. with abnormal values	No. with abnormal values
Protein C antigen	9 (45%)*	0
Protein C function	8 (40%)†	0
Protein S total	0	0
Protein S free	0	1 (5%)‡
Antithrombin	2 (10%)‡	0

The cut-off levels for the lower limit of normal for protein C, protein S, and antithrombin were derived from a study of healthy children and set at ≤ 5 th percentile for each of 4 non-overlapping age groups: 6 months to 1 year; 1 to 5.5 years; 5.5 to 11.2 years; and 11.2 to 16.9 years (see Methods).

* $P = .001$, Fisher exact test.
† $P = .002$, Fisher exact test.
‡Not significant ($P > .05$, Fisher exact test).

III chromogenic assay kit (Biopool, Burlington, Ontario, Canada). Factor X antigen level was measured by Laurell-type electroimmunoassay with polyclonal specific antibody (Celsus Laboratories, Inc, Cincinnati, Ohio). D-Dimer level was measured by ELISA with the Instant I.A. D-dimer kit (Diagnostica Stago).

The distribution of the data was analyzed for normality. A Fisher exact test was used to compare the proportion of subjects in group I who had abnormal coagulation test results, which defined the contingency table, with subjects in group II. Correlations were analyzed by the non-parametric Kendall rank order test.

RESULTS

Group I

The 20 subjects receiving VPA therapy for seizure disorders ranged in age from 6 months to 12 years (mean age, 6.8 years; median, 7.9 years). They had been receiving treatment for periods ranging from 2 months to 7 years (median, 18 months). No other anticonvulsants were being taken at the time the studies were performed. The only other medication being given was levo-

carnitine (2 subjects). None of the subjects were receiving antibiotic therapy. One subject had a history of meningitis, one had a history of prematurity and intraventricular hemorrhage, two had cerebral palsy, and one had Rett syndrome. None had known liver disease. One child was receiving feedings by nasogastric tube because of feeding problems and one had a history of recurrent upper respiratory tract infections. None of the study subjects had a history suggestive of venous thromboembolism or stroke.

Group II

The 20 subjects receiving anticonvulsant therapy other than VPA ranged in age from 2 to 12 years (mean age, 7.9 years; median, 7.9 years). One subject had a history of congenital cytomegalovirus infection, 2 had a history of lead poisoning, 4 had cerebral palsy, 2 had hydrocephalus with ventriculo-peritoneal shunts, and 1 had congenital heart disease, which had been surgically corrected. None of the subjects had received VPA therapy for at least 4 months. The numbers of subjects receiving various anticonvulsants are as follows: carbamazepine (n = 11), lamotrigine (n = 3), phenytoin (n = 2),

topiramate (n = 1), lamotrigine and gabapentin (n = 1), lamotrigine and carbamazepine (n = 1), and lamotrigine and clonazepam (n = 1). Other medications included metoclopramide (n = 1) and imipramine (n = 1). None were receiving antibiotic therapy.

VPA Levels, Platelet Counts, and Fibrinogen Levels

Subjects in group I had VPA levels that ranged from 9.3 to 170 $\mu\text{g}/\text{mL}$ (median level, 104 $\mu\text{g}/\text{mL}$). In all but one patient VPA dosage had been constant for at least 8 weeks before blood was obtained for this study. Fibrinogen levels were $< 150 \text{ mg}/\text{dL}$ in 2 group I subjects (VPA levels 125 and 150 $\mu\text{g}/\text{mL}$). Platelet counts were decreased ($41, 55, 44, 134 \times 10^3/\mu\text{L}$) in 4 group I subjects, 2 of whom had levels of VPA $> 100 \mu\text{g}/\text{mL}$. One of the other 4 subjects with a low platelet count was admitted to the hospital for treatment of epistaxis and seizure, at which time the platelet count was $19 \times 10^3/\mu\text{L}$ and VPA level was 256 $\mu\text{g}/\text{mL}$. VPA was withheld for 2 days. The platelet count increased to $41 \times 10^3/\mu\text{L}$, and the VPA level decreased to 25.6 $\mu\text{g}/\text{mL}$, at which time the subject was enrolled in the VPA study. No other subjects had a history of recent bleeding problems. All subjects in group II had normal platelet counts. One subject in group II had a fibrinogen level of 120 mg/dL .

Protein C, Protein S, and Antithrombin Levels

The levels of protein C antigen, protein C activity, fibrinogen, and platelets in groups I and II are compared in the Figure. An abnormal level for the coagulant and anticoagulant proteins was defined as ≤ 5 th percentile for healthy children in the respective age group (see Methods). The numbers of subjects in groups I and II with abnormally low levels of the anticoagulant proteins are compared in the Table. Factor X levels were measured

to rule out the effect of VPA on the vitamin K-dependent proteins. Normal levels were observed in all subjects in groups I and II. Normal alanine aminotransferase levels and normal prothrombin and activated partial thromboplastin times were observed in all subjects in both groups, providing additional evidence against vitamin K deficiency or liver dysfunction. D-Dimer levels were determined to rule out intravascular coagulation as a cause for decreased protein C levels. D-Dimer levels were undetectable in 18 of 20 VPA-treated subjects and borderline (0.5-1.0 µg/mL) in 2 subjects.

In the group I subjects, protein C antigen levels tended to inversely correlate with VPA levels, but this association was weak ($r = -0.27968$, $P = .0854$). The protein C activity levels were more strongly correlated with VPA levels ($r = -35545$, $P = .0295$).

DISCUSSION

Patients receiving VPA have been reported to have decreased platelet counts, altered platelet aggregation, and prolonged bleeding times.¹⁻⁵ The degrees of platelet count suppression and bleeding time prolongation correlate with VPA plasma concentrations.^{3,5} Hypofibrinogenemia has also been reported in association with VPA therapy, although the mechanism is unknown.^{6,7} Low fibrinogen levels have not been related to liver disease or disseminated intravascular coagulation. Increased fibrinolysis caused by VPA is unlikely, because elevated levels of fibrinogen degradation products or D-dimer have not been reported. A rapid rise in fibrinogen level follows discontinuation of the drug.

We found reduced protein C levels in up to 45% of VPA-treated children. The association of VPA therapy and protein C deficiency has not been previously reported. Acquired protein C deficiency may be seen in liver disease, vitamin K deficiency states, thrombosis, or disseminated intravascular coagulation.⁸

The normal alanine aminotransferase levels, the normal coagulation study findings (prothrombin and activated partial thromboplastin time), and the normal levels of protein S and coagulation factor X provide evidence for normal liver function. Similarly, normal coagulation study results in association with normal levels of the vitamin K-dependent coagulation factors X and protein S provide evidence that vitamin K deficiency is not the cause of the low protein C levels. Normal levels of D-dimer were present in all but 2 patients receiving VPA therapy. The 2 patients with borderline elevations of D-dimer had normal protein C levels, suggesting that low-grade intravascular coagulation was not a cause of VPA-related protein C deficiency. Whether protein C deficiency secondary to VPA therapy is due to increased turnover or decreased production of the protein must await further studies.

The protein C level in some of our study subjects was reduced to a level that has been reported to be associated with thromboembolic complications, including stroke, in children.⁹⁻¹¹ Whether the reduced protein C level in our patient was causally related to her stroke, however, must remain speculative, because to date, there are no other reports of VPA therapy associated with thromboembolic disease. The lack of reports of thrombosis associated with VPA therapy is puzzling, considering the high incidence of protein C deficiency associated with its use. The apparent rarity of thrombosis associated with VPA therapy may be due to the failure to recognize the association. On the other hand, low levels of protein C may not be sufficient to induce thrombosis unless other prothrombotic factors are present. Particularly in children, thrombosis is a multi-causal disease, and inherited and acquired risk factors are often simultaneously present.¹² In some instances, the presence of multiple risk factors has supra-additive effects, with the relative thrombotic risk of the combined risk

factors being greater than the sum of the relative risks of the individual factors.¹² Besides deficiencies of antithrombin, protein C, and protein S, other common genetic risk factors for thrombosis include the factor V Leiden mutation and the 20210A mutation in the 3'-untranslated region of the prothrombin gene.¹² The factor V Leiden mutation causes resistance to activated protein C. A thorough family history may aid in the recognition of the familial thrombophilic disorders. Common causes of acquired thrombophilic abnormalities in children include malignancy, infection, surgery, trauma, central venous catheters, and the presence of antiphospholipid antibody.^{10,11} We believe pediatricians should be aware of the possibility of hypercoagulability in children receiving VPA therapy, particularly in children with a family history of thrombosis. Unless other reports of VPA therapy associated with thromboembolic complications appear, we believe it is premature to suggest monitoring protein C levels during therapy. However, we would suggest an evaluation of protein C levels in any child suspected of having thrombotic complications while receiving VPA. Larger studies are needed to confirm our findings and further define the mechanism underlying acquired protein C deficiency related to VPA.

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