CHANGES IN HOMOCYSTEINE LEVELS IN EPILEPTIC CHILDREN RECEIVING SODIUM VALPROATE
Sodyum valproat alan epileptik çocuklarda homosistein düzeylerindeki değişiklikler

Elif OZEROL[1], İbrahim OZEROL[2], Mehmet ASLAN[3], Cengiz YAKINCI[4], Ismail TEMEL[5]

Abstract

Purpose: *The data regarding valproate and their influence on folate and homocysteine levels are conflicting. The aim of this study was to evaluate whether differences exist in homocysteine, folate, and vitamin B_{12} levels in children receiving valproate.*

Patients and Methods: *Sixty-five patients receiving sodium valproate and twenty-five healthy controls were included in the study. Serum total homocysteine levels were analyzed by enzyme linked immunosorbent assay kit. Vitamin B_{12} and folate values were measured by Bio DPC kits.*

Results: *Serum homocysteine concentrations were significantly higher in patients than those of the controls. Moreover, serum folate levels were observed to be significantly decreased in patients compared to controls. On the contrary, serum vitamin B_{12} levels did not change in the patients.*

Conclusion: *Our data show that prolonged treatment with valproate increases serum homocysteine concentrations suggesting that an effect of inducer valproate on homocysteine metabolism could exist, which is not independent of the effect of low folate levels. These results indicate that homocysteine levels should be determined in order to identify hyperhomocysteinemia among patients receiving sodium valproate.*

Key Words: *Epilepsy, Folic acid, Homocysteine, Valproate*

Özet:

Amaç: *Valproatın folat ve homosistein düzeyleri üzerinde etkileri ile ilgili veriler çelişkilidir. Bu çalışmanın amacı valproat alan çocuklarda homosistein, folat ve vitamin B_{12} düzeylerinde farklılık olup olmadığını değerlendirmektir.*

Hastalar ve Yöntem: *Sodyum valproat alan altmışbeş hasta ve yirmibeş sağlıklı kontrol çalışıldı. Serum total homosistein düzeyleri ELİZA kitiyle analiz edildi. Vitamin B_{12} and folat değerleri Bio DPC kitleriyle ölçüldü.*

Bulgular: *Serum Hcy konsantrasyonları hastalarda kontrollerdekinden anlamlı olarak daha yüksekti. Üstelik, kontrollerle karşılaştırıldığında serum folat düzeylerinin hastalarda anlamlı olarak azaldığı gözlendi. Bunun aksine, serum vitamin B_{12} düzeyleri değişmedi.*

Sonuç: *Sonuçta, bizim verilerimiz valproat ile uzun süre tedavinin serum homosistein konsantrasyonlarını arttırdığını göstermektedir; homosistein metabolizması üzerinde valproatın indükleyici bir etkisi olabilir ve bu etki düşük folat düzeylerinden bağımsız değildir. Bu sonuçlar sodium valproat alan hastalarda hiperhomosisteineminin tanımlanması için homosistein düzeylerinin tayin edilmesi gerektiğini göstermektedir.*

Anahtar Kelimeler: *Epilepsi, Folik asit homosistein, Valproat*

In recent years, plasma total homocysteine (tHcy) level and its association with cardiovascular diseases have received considerable attention. Vitamin B_{12} and folate function as coenzymes in the metabolism of Hcy. It is suggested that deficiency of these vitamins is associated with increased plasma concentrations of Hcy (1, 2). Ono reported that 4 of 130 epileptic patients receiving various AEDs had low plasma folate and elevated Hcy concentrations (3). Schwanninger found that mean plasma Hcy concentrations were significantly increased in a total of 51 patients receiving monotherapy or various combinations of AEDs as compared to controls (4). More recently, Yoo and Hong reported that approximately 25% of patients receiving AEDs had hyperhomocysteinemia (5). Apeland confirmed these observations but demonstrated that AEDs are, by themselves, independent predictors of a high plasma Hcy level (6).

İnönü Üniversitesi Turgut Özal Tıp Merkezi 44069 MALATYA Biyokimya.Uzm.Dr.[1], Doç.Dr.[5]. Mikrobiyoloji. Doç.Dr.[2]. Pediatri.Araş.Gör.Dr.[3], Prof.Dr.[4].

Geliş tarihi: 15 Ocak 2003

As to the effect of AEDs therapy on vitamin B_{12} or vitamin B_6 nutritional status, no consensus has been achieved in the literature (7-10). Furthermore, reports on the effect of AEDs on Hcy concentrations are scarce. Elevated plasma homocysteine concentration has been associated with an increased risk for occlusive vascular disease. Hyperhomocysteinemia may be caused by the deficiency of one of the vitamins or the combination, and these deficiencies have been frequently associated with the administration of AEDs. We hypothesized that hyperhomocysteinemia exists in patients receiving AED; thus, a long-term administration of these drugs possibly results in an increased risk of occlusive vascular disease. To test this hypothesis, we undertook the study presented here to evaluate whether differences exist in Hcy serum concentrations in children receiving VPA, monotherapy, in comparison with healthy control subjects and to determine the possible relationship between Hcy levels and folate, vitamin B_{12} serum concentrations. For this purpose, we measured serum concentrations of Hcy, folate, and vitamin B_{12} in outpatients with epilepsy who were treated with VPA and in healthy controls.

MATERIALS AND METHODS

We studied 65 patients, aged from 0,583 to 13 years, suffering from various types of epilepsy (38 tonic-clonic, 15 myoclonic-astatic, 7 tonic, and 5 clonic) and 25 healthy subjects, aged from 0 to 15 years. Duration of the illness (year) was 3.7±1.5 (mean± SEM). They did not suffer from any other disease. Fasting blood samples obtained from subjects in the morning. After coagulation, the blood samples were centrifuged, separated, and stored at-20 ^oC on the day of sample collection.Hcy, vitamin B_{12} and folate were investigated in the serum. Serum tHcy levels were analyzed by enzyme linked immunosorbent assay (ELISA) kit (Homocysteine Enzyme Immunoassay Kit, Bio-Rad Lab, Oslo, Norway). All samples were assayed in duplicate. Vitamin B_{12} and folate values were measured by Bio DPC kits using Immulite 2000 instrument with Chemiluminescence method. Since the blood samples used in this study were collected for routine blood analysis, additional consent from patients and clearance by the Ethics committee were not required.

Statistical analysis: *Data were analyzed by using the SPSS for Windows computing program. Results were expressed as mean ± SEM. P values < 0.05 were regarded as statistically significant.*

RESULTS

Brief statistics for homocysteine, folate and vitamin B_{12} levels are presented in Table 1. Serum Hcy concentrations were significantly higher in epileptic children than in healthy controls ($p<0.001$). However, serum folate levels were significantly lower in VPA receiving group than in controls ($p<0.001$). The mean vitamin B_{12} concentrations were found to be unchanged in patients compared to controls. In correlation analyses, in patients with epilepsy receiving VPA treatment total Hcy had significantly negative correlations with folate levels.

DISCUSSION

Table 1. *Homocysteine, folate and vitamin B_{12} concentrations in serum from patients with epilepsy and healthy control subjects*

	Epilepsy (n=65) mean±SEM	Healthy controls (n= 25) mean±SEM	p
Folate (nmol/L)	5.1 ± 2.4	10.4 ± 3.5	<0.001
Vitamin B_{12} (nmol/L)	619 ± 282	799 ± 285	NS
tHcy (µmol/L)	17.6 ± 8.9	9.4 ± 6.5	<0.001
Valproate (µg/mL)	51.2 ± 44.7	---	

Data from several investigations demonstrates that VPA is associated with either low or normal folate levels, either high or normal plasma tHcy levels (6, 12-15). A fasting plasma tHcy concentration above 15 mmol/L is the most common definition of hyperhomocysteinemia (16), which is associated with approximately a three-fold increased risk of myocardial infarction. For these reasons, the administration of folate is recommended by Ono (3) in epileptic patients with hyperhomocysteinemia due to folate deficiency to prevent vascular disease. The current study shows that epileptic patients receiving VPA have increased plasma levels of Hcy. Our results are in agreement with a previous report on Hcy concentrations in patients with epilepsy (12).

The increased Hcy concentration may be due to a deficiency in vitamins necessary for the metabolism of Hcy. On the other hand, high plasma levels of Hcy could be related to the presence of epilepsy. However, the baseline evaluation allows excluding the possibility that this abnormality could be a result of the convulsive disorder itself or other situations (e.g. genetic abnormalities of metabolism of Hcy).

In order to better understand the metabolism of Hcy, we also studied the levels of serum vitamin B_{12} and serum folate. We found significantly lower serum folate concentrations in patients with epilepsy than healthy controls. However, there was no difference in concentrations of vitamin B_{12} between patients and controls.

The mechanisms by which AEDs alter folate metabolism are still uncertain. Data on VPA effects on folic acid are conflicting. The present study shows that there was a decrease of serum folate levels in patients being treated with VPA. Contrary to our findings, Apeland reports that valproate does not influence folate and Hcy levels in adult patients (6). Wegener and Nau described that VPA increased the concentrations of folate (17). The effect of valproate on plasma folate concentrations as shown by this study is similar to that by Verrotti (12). Their patients on VPA had high plasma tHcy and low folate levels. The reason for the difference between these findings is unknown. It may be possible to attribute it to the difference in the practice of folic acid fortification for commonly consumed foods between the countries. As all study subjects were from the same cultural and geographic region, we believe that there were only minor dietary differences between patients and controls. However, it may be a correlation between folate levels and drug dosage or duration of treatment. Serum folate levels were not correlated with serum concentrations of VPA. Our data indicate that the result of long-term treatment with VPA may exert a major influence on serum folate levels.

The literature on the vitamin B_{12} status in patients undergoing treatment with VPA is controversial. Decreased, normal and increased vitamin B_{12} concentrations have been reported in patients with epilepsy (11-13). We found no difference in concentrations of vitamin B_{12} between patients and controls. This study did not confirm the previous finding that patients on VPA have elevated vitamin B_{12} levels in serum (9, 10, 13).

In conclusion, our findings suggest that VPA play a critical role in hyperhomocysteinemia and the folate depletion seen in patients receiving VPA. Thus, a long-term administration of these drug possibly results in an increased risk of occlusive vascular disease.

These results indicate that homocysteine levels should be determined in order to identify hyperhomocysteinemia among patients receiving sodium valproate. These patients may have a higher folate requirement to maintain a normal homocysteine level because of a nutritional deficiency of vitamin B_{12} or folate leads to increased homocysteine concentrations.

REFERENCES

1. Ubbink JB, Vermaak WJH, Van der Merwe A, Becker PJ. Vitamin B12, Vitamin B6 and folate nutritional status in men with hyperhomocysteinemia. Am J Clin Nutr 1993; 57: 47-53.
2. Ubbink JB, Van der Merwe A, Delport R, et al. The effect of a subnormal vitamin B6 status on homocysteine metabolism. J Clin Invest 1996; 98: 177-184.
3. Ono H, Sakamoto A, Eguchi T, et al. Plasma total homocysteine concentrations in epileptic patients taking anticonvulsants. Metabolism 1997; 46: 959-962.
4. Schwaninger M, Ringleb P, Winter R, et al. Elevated plasma concentrations of homocysteine in antiepileptic drug treatment. Epilepsia 1999; 40: 345-350.
5. Yoo J-H, Hong SB. A common mutation in the methylenetetrahydrofolate reductase gene is a determinant of hyperhomocysteinemia in epileptic patients receiving anticonvulsants. Metabolism 1999; 48: 1047-1051.
6. Apeland T, Mansoor MA, Strandjord RE. Antiepileptic drugs as independent predictors of plasma total homocysteine levels. Epilepsy Res 2001; 47: 27-36.
7. Malpas JS, Spray GH, Witts LJ. Serum folic-acid and vitamin-B12 levels in anticonvulsant therapy. Br Med J 1966; I: 955-957.
8. Preece J, Reynolds EH, Johnson AL. Relation of serum to red cell folate concentrations in drug-treated epileptic patients. Epilepsia 1971; 12: 335-340.
9. Dastur DK, Dave UP. Effect of prolonged anticonvulsant medication in epileptic patients: serum lipids, vitamins B6, B12, and folic acid, proteins, and fine structure of liver. Epilepsia 1987; 28: 147-159.
10. Hauser E, Seidl R, Freilinger M, Male C, Herkner K. Hematologic manifestations and impaired liver synthetic function during valproate monotherapy. Brain Dev 1996; 18: 105-109.
11. Kishi T, Fujita N, Eguchi T, Ueda K. Mechanism for reduction of serum folate by antiepileptic drugs during prolonged therapy. J Neurol Sci 1997; 145: 109-112.
12. Verroti A, Pascarella R, Trotta D, Giuva T, Morgese G, Chiarelli F. Hyperhomocysteinemia in children treated with sodium valproate and carbamazepine. Epilepsy Res 2000; 41: 253-257.
13. Tamura T, Aiso K, Johnston KE, Black L, Faught E. Homocysteine, folate, vitamin B-12 and vitamin B-6 in patients receiving antiepileptic drug monotherapy. Epilepsy Res 2000; 40: 7-15.
14. Krause KH, Bonjour JP, Berlit P, Kynast G, Schmidt-Gayk H, Schellenberg B. Effect of long-term tratment with antiepileptic drugs on the vitamin status. Drug Nutr Interact 1988; 5: 317-343.
15. Froscher W, Maier V, Laage M, et al. Folate deficiency, anticonvulsant drugs and psychiatric morbidity. Clin Neuropharmacol 1995; 18: 165-182.
16. Kang SS, Wong PW, Malinow MR. Hyperhomocysteinemia as a risk factor for occlusive vascular disease. Ann Rev Nutr 1992; 12: 279-298.
17. Wegner C, Nau H. Alteration of embryonic folate metabolism by valproic acid during organogenesis: implications for mechanism of teratogenesis. Neurology 1992; 42: 17-24.