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ANALYSIS OF ACYLCARNITINE PROFILES IN CHILDREN WITH IDIOPATHIC EPILEPSY USING VALPROIC ACID

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Purpose: To evaluate whether valproic acid (VPA) treatment in children with epilepsy affects the plasma fatty acylcarnitine esters.

Materials and Methods: Fifty children (mean age, 10±3.9) with epilepsy receiving VPA treatment for at least one year were evaluated along with 110 children of similar age as a control group. Levels of free carnitine and acylcarnitine profiles were studied with tandem mass spectrometry (Tandem-MS).

Results: Free carnitine levels were within the normal limits. Valproic acid was found to affect the levels of the following individual acylcarnitines (medians): C4–acylcarnitine (0.56 µmol/L; controls 0.39 µmol/L), 3-hydroxy-isovalerylcarnitine (C5-OH) (0.23 µmol/L; controls 0.14 µmol/L), C14-acylcarnitine (0.11 µmol/L; controls 0.16 µmol/L), C16-acylcarnitine (0.76 µmol/L; controls 2.5 µmol/L), C16-OH-acylcarnitine (0.04 µmol/L; controls 0.07µmol/L), and C18:1-acylcarnitine (0.8 µmol/L; controls 0.97 µmol/L). A statistical comparison of these acylcarnitines between subjects and controls showed a statistically significant effect ($p<0.05$).

Conclusion: The detection of normal free carnitine levels, the detection of 5-OH isovaleryl carnitine higher than other carnitines, and the normally found levels of some acylcarnitine fractions led us to conclude that VPA does not have a significant effect on the carnitine and acylcarnitine metabolism as a drug side effect when the carnitine metabolism is considered. Therefore, we think that a study of the acylcarnitine profile in addition to the detection of the levels of free carnitine is essential in understanding the exact effect of VPA on the carnitine metabolism.

Key Words: Acylcarnitine, Antiepileptics, Children.

VALPROİK ASİT KULLANAN İDİYOPATİK EPİLEPSİLİ ÇOCUKLARDA AÇİL KARNİTİN PROFİLLERİNİN DEĞERLENDİRİLMESİ

Amaç: Valproik asit (VPA) tedavisi alan epilepsili çocuklarda plazma açilkarnitin düzeylerinin etkilenip etkilenmeyeceğini araştırmak amaçlı yapılmıştır.

Materyals ve Metods: En az bir yıldır VPA tedavisi alan 50 epilepsili çocuk (ortalama yaş, 10±3.9) ve benzer yaştaki 110 çocuk control grubu olarak incelenmiştir. Serbest karnitin ve açilkarnitin düzeyleri Tandem Mass Spectrometry (Tandem-MS) yöntemi kullanılarak ölçülmüştür.

Sonuçlar: Serbest karnitin düzeyleri normal olarak saptanmıştır. Valproik asitin aşağıda belirtilen açilkarnitin düzeylerini kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı bir şekilde etkilediği ($p<0.05$) saptanmıştır (median değerler): C4–açilkarnitin (0.56 µmol/L; kontrol, 0.39 µmol/L), 3-hidroksi-isovalerilkarnitin (C5-OH) (0.23 µmol/L; kontrol, 0.14 µmol/L), C14-açilkarnitin (0.11 µmol/L; kontrol, 0.16 µmol/L), C16-açilkarnitin (0.76 µmol/L; kontrol, 2.5 µmol/L), C16-OH-açilkarnitin (0.04 µmol/L; kontrol, 0.07 µmol/L), C18:1-açilkarnitin (0.8 µmol/L; kontrol, 0.97 µmol/L).

Tartışma: Serbest karnitin düzeylerinin normal, 5OH izovaleril karnitin düzeylerinin diğer açilkarnitinlerden yüksek ve bazı açil karnitin düzeylerinin sınırda düşük olmas, karnitin metabolizması göz önüne alındığında bir ilaç yan etkisi olarak VPA'nın sebest karnitin ve açilkarnitin metabolizmasında çok önemli bir etkisi olmadığını düşündürmüştür. Bu nedenle VPA'nın çocuklardaki karnitin metabolizmasına tam etkisinin saptanması için serbest karnitin yanı sıra açilkarnitin profilleri için ayrıntılı ve ek çalışmalara ihtiyaç olduğu düşüncesindeyiz.

Anahtar Kelimeler: Açilkarnitin, Antiepileptik İlaçlar, Çocuklar.

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INTRODUCTION

Valproic acid (2-n-propylpentanoic acid, a short-chain fatty acid, VPA) is a broad-spectrum antiepileptic drug commonly used in children. (1-3). Besides its adverse effects, such as behavioral changes, alopecia, tremor, increased appetite, or gastrointestinal disturbances, it also has some rare but serious side effects such as Reye-like syndrome, or irreversible hepatotoxicity. Valproic acid causes carnitine deficiency either by inhibiting the β-oxidation of fatty acids in the liver, by its direct toxic effects on liver mitochondria, or by conjugating with acylcarnitine transferase to form valproilcarnitine, which is excreted in the urine (1-10). Although a decrease in serum concentration of carnitine related to VPA treatment has been repeatedly reported, there exist contradictory studies about the effects of VPA on the carnitine metabolism (3,4,6,8-12).

Carnitine can be obtained from the diet, and it can also be synthesized from trimethyl-lysine (6). It is necessary for the transport of long-chain fatty acids from the cytoplasm into the mitochondria for β-oxidation (1,9,10). It serves for the transport of short-chain acyl compounds from the mitochondria into the cytoplasm, for the regulation of the cytosolic acyl CoA/CoA ratio, and for the removal of the toxic esters of acylcarnitine and their metabolites from the mitochondrium (1).

In most studies, the fractions of acylcarnitine were globally assessed, and only a few studies displayed little differences between the short and long chain acylcarnitines (2,6,8,13). In addition, there exists no comprehensive study examining the effects of VPA on the mechanism of acylcarnitine in pediatric subjects.

The aim of this study was to evaluate whether VPA treatment in children with epilepsy affects the plasma fatty acylcarnitine esters, using the quantitative analysis of human plasma acylcarnitines by LC-MS/MS.

MATERIALS AND METHODS

Our study comprised 50 children with epilepsy admitted to the Department of Pediatric Neurology at Gazi University Faculty of Medicine as outpatients and receiving VPA monotherapy at least for one year, and 110 children of similar age as a control group. The patients were between 4 and 16 years old (mean age, 10±3.9); 29 of them were female, and 21 male.

Valproic acid dosages were on average 20.86±3.62 mg/kg per day (min 10, max: 30 mg/kg per day), and valproic acid levels of all the patients were within the therapeutic ranges (63.42±9.83 µg/dl). In our laboratory, therapeutic ranges of VPA are 50-75 µg/dl.

Levels of free carnitine and acylcarnitine in the spots of blood specimens of the patient and control groups, which were obtai-

Table 1: Levels of free carnitine and acylcarnitine levels in the valproic acid and control groups.

Free and Acylcarnitines (µmol/L)	Patients[a] (n:50)	Controls[a] (n:110)
Free carnitine	28.3 (10.4-70.4)	21,2 (11.5-91.7)
C2	20.6 (8.6-45.5)	21,0 (2.7-64.5)
C3	1.84 (0.6-4.3)	1.79 (0.2-9.6)
C4	0.56 (0.14-5.12)	0.39 (0.13-2.04)[b]
C5:1	0.05 (0.01-0.34)	0.06 (0.0-0.38)
C5	0.16 (0.08-0.5)	0.15 (0.05-0.49)
Butyryl carnitine	0.1 (0.03-0.4)	0.12 (0.02-0.39)[b]
C6	0.19 (0.03-1.89)	0.13 (0.02-0.89)
C5-OH	0.23 (0.05-0.71)	0.14 (0.04-0.9)[b]
C8	0.09 (0.01-0.26)	0.08 (0.01-0.95)
C10	0.13 (0.04-0.39)	0.11 (0.0-0.38)
Glutaryl carnitine	0.06 (0.01-0.36)	0.07 (0.0- 0.25)
C12	0.11 (0.01-0.43)	0.13 (0.0-0.48)
Methyl-glutaryl carnitine	0.05 (0.0-0.19)	0.06 (0.0-2.43)
C14:1	0.08 (0.0-0.45)	0.09 (0.0-0.33)
C14	0.11 (0.0-0.8)	0.16 (0.0-0.54)[b]
C14-OH	0.03 (0.0-0.21)	0.03 (0.0-0.3)
C16	0.76 (0.3-1.6)	2.5 (0.12-6.8)[b]
C16:1-OH	0.08 (0.03-0.49)	0.09 (0.0-10.1)
C16-OH	0.04 (0.01-1.12)	0.07 (0.0-0.29)[b]
C18:1	0.8 (0.29-1.76)	0.97 (0.15-2.47)[b]
C18:1-OH	0.05 (0.0-015)	0.05 (0.0-0.16)

a: Medians (minimum-maximum)

b: $p < 0.05$:Mann-Whitney U test; compared between children taking VPA and controls.

ned on Guthrie cards 12 hours after the last drug dose, were studied with tandem mass spectrometry (Tandem-MS), a method suggested by Chace et al. (14). The results were given as µmol/L.

While medians, minimum and maximum levels are presented for descriptive purposes, two-group comparisons were done using an unpaired Mann-Whitney U test of the abnormal distribution of the VPA group. A p value less than 0.05 was considered significant.

Approval for this study was given by Gazi University Faculty of Medicine Ethical Committee.

RESULTS

While a significant rise in C5-OH carnitine levels was detected in children taking VPA as compared with the control group ($p<0.05$), free carnitine levels were within the normal reference values in both groups. The main quantitative results of free carnitine and acylcarnitine profiles are shown in Table 1.

Valproic acid was found to affect the levels of the following individual acylcarnitines (medians): C4–acylcarnitine (0.56 µmol/L; controls 0.39 µmol/L), 3-hydroxy-isovalerylcarnitine (C5-OH) (0.23 µmol/L; controls 0.14 µmol/L), C14-acylcarnitine (0.11 µmol/L; controls 0.16 µmol/L), C16-acylcarnitine (0.76 µmol/L; controls 2.5 µmol/L), C16-OH-acylcarnitine (0.04 µmol/L; controls 0.07 µmol/L), and C18:1-acylcarnitine (0.8 µmol/L; controls 0.97 µmol/L). A statistical comparison of these acylcarnitines between subjects and controls showed a statistically significant effect ($p<0.05$). The altered levels mentioned above are given highlighted, and the concentrations of all the remaining acylcarnitines were within their normal reference ranges.

DISCUSSION

Numerous studies have been conducted on the effects of valproic acid as well as the other antiepileptic drugs on carnitine concentrations (5,6,9-12). Similar to previous studies on adult patients using VPA (6,13,15), in our study, free carnitine levels were within the normal range, whereas 3-OH isovaleryl carnitine (C5-OH) levels in the patient group were considerably higher than those in the control group. In a study conducted by Silva et al. on adult patients receiving VPA monotherapy, serum levels of total or free carnitine remained unaltered while 3-OH isovaleryl carnitine levels were significantly higher in the patient group when compared with the control group (13). These findings led us to conclude that VPA or its metabolites might cause direct toxic effects on liver mitochondria, or might be capable of influencing the leucine metabolism by inhibiting

3-methylcrotonyl CoA carboxylase, biotinidase, and holocarboxylase synthase enzymes, thereby giving rise to an increase in the 3-hydroxy-isovalerylcarnitine fraction (6,13). Patients with genetic deficiencies in these three enzymes present an elevated excretion of 3-hydroxy-isovaleric acid, together with other secondary and pathognomic metabolites. 3-Hydroxy-isovaleric acid has recently been reported to be a deranged mitochondrial respiratory chain. It remains to be established whether this association (and that with 3-hydroxy-isovaleryl–carnitine) also holds true for VPA-treated patients (13).

Riva et al., when assessing epileptic patients before and after VPA treatment, demonstrated that the serum levels as well as urinary excretion of free carnitine had considerably decreased, and that the serum levels and urinary excretion of acylcarnitine in contrast had elevated (7). It was stressed that in rats given VPA, similarly, free serum levels were decreased while acylcarnitine levels were elevated, and that morphologically pathologic enlargements were observed in the liver mitochondria (4,16,17). In studies conducted on rats and humans, it was underlined that VPA enhanced the excretion of acylcarnitines from the kidneys as well as diminishing their reabsorption even a short period after its administration, thus enhancing the conversion of carnitine to acylcarnitine, which in turn results in carnitine deficiency (3,5,11,12,17).

Although in some publications carnitine supplementation during VPA treatment was shown to be useful (1,3,4,12,13,16), some studies revealed no statistically significant difference between the patients treated with and without VPA concerning their carnitine concentrations (1,6,13). It was reported that the decreased levels of free serum carnitine as well as the increased levels of acylcarnitine returned to normal in rats that had undergone carnitine therapy after VPA administration (4). It is also reported that while carnitine deficiency may occur as a result of VPA treatment, it may also occur due to decreased carnitine intake from the diet (1,6). Thus, there is no agreement concerning the administration of carnitine to patients receiving VPA treatment (13).

In the literature, there is no other comprehensive study examining the profile in the pediatric age group except for some studies concerning the levels of free carnitine and total carnitine (1,8,15,17). In our study, although we found a decrease, which was minimal, in some acylcarnitine fractions statistically significant, it is not important as a drug side effect in terms of the carnitine metabolism. Some studies reported that serum total and free carnitine levels were significantly lower in pediatric patients receiving VPA monotherapy or polytherapy, and that this rate was much lower in the polytherapy group (1,8,15,17). These investigators emphasized that the interaction between the drugs enhanced the metabolism of VPA, thereby causing an additional diminution in carnitine levels. Besides some studies showing that there is an inverse relationship between the duration of VPA usage and carnitine levels but no correlation between the VPA dose and carnitine deficiency (1,2), some other studies report that VPA impairs the carnitine metabolism, and that it generates a dose-related increase in serum ammonia (3,6,9,11).

In our study, the detection of normal free carnitine levels, of 5-OH isovaleryl carnitine being higher than other carnitines and of normal levels of some acylcarnitine fractions led us to conclude that VPA does not have a significant effect on the carnitine and acylcarnitine metabolism. Therefore, we think that an additional study of acylcarnitine profile beside detection of the levels of free carnitine is essential to understand the exact effect of VPA on the carnitine metabolism.

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REFERENCES

1. Chung S, Choi J, Hyun T. Alterations in the carnitine metabolism in epileptic children treated with valproic acid. J Korean Med Sci 1997; 12: 553-558.
2. Segade SR, Pena CA, Tutor JC. Carnitine deficiency associated with anticonvulsant therapy. Clin Chim Acta 1989; 181: 175-182.
3. Arrigoni ME, Caso V. Carnitine protects mitochondria and removes toxic acyls from xenobiotics. Drugs Exp Clin Res 2001; 27: 27-49.
4. Nishida N, Sugimoto T, Araki A. Carnitine metabolism in valproate-treated rats: the effect of L-carnitine supplementation. Ped Res 1987; 22: 500-503.
5. Camina MF, Rozas I, Gomez JM. Short-term effects of administration of anticonvulsant drugs on free carnitine and acylcarnitine in mouse serum and tissues. Br J Pharmacol 1991; 103: 179-1183.
6. Beghi E, Bizzi A, Codegoni. Valproate, carnitine metabolism, and biochemical indicators of liver function. Epilepsia 1990; 31: 346-352.
7. Riva R, Albani F, Gobbi G. Carnitine disposition before and during valproate therapy in patients with epilepsy. Epilepsia 1993; 34: 184-187.
8. Laub MC, Brunner IP, Jaeger G. Serum carnitine during valproic acid therapy. Epilepsia 1986; 27: 559-562.
9. Eyer F, Felgenhauer N, Gempel K. Acute valproate poisoning: pharmacokinetics, alteration in fatty acid metabolism, and changes during therapy. J Clin Psychopharmacol 2005; 25: 376-380.
10. Silva MF, Jakobs C, Duran M. Valproate induces in vitro accumulation of long-chain fatty acylcarnitines. Mol Genet Metab 2001; 73: 358-361.
11. Matsuda I, Ohtani Y, Ninomiya N. Renal handling of carnitine in children with carnitine deficiency and hyperamonemia associated with valproate therapy. J Pediatr 1986; 109: 131-134.
12. Camina MF, Rozas I, Gago C. Alteration of renal carnitine metabolism by anticonvulsant treatment. Neurol 1991; 41: 1444-1448.
13. Silva MFB, Selhorst J, Overmans H. Characterization of plasma acylcarnitines in patients under valproate monotherapy using ESI-MS/MS. Clin Biochem 2001; 34: 635-638.
14. Chace DH, Hillman SL, Va Hove JLK, Naylor EW. Rapid diagnosis of MCAD deficiency: quantative analysis of octanocarnitines in newborn blood spots by tandem mass spectrometry. Clin Chem 1997; 43: 2160-2113.
15. Moreno FA, Macey H, Schreiber B. Carnitine levels in valproic acid-treated psychiatric patients: a cross-sectional study. J Clin Psychiatry 2005; 66: 555-558.
16. Murakami K, Sugimoto T, Nishida N. Carnitine metabolism and morphometric change of liver mitochondria in valproate-treated rats. Neuropediatrics 1990; 21: 187-190.
17. Sugimoto T, Woo M, Nishida N. Hepatotoxicity in rat following administration of valproic acid. Epilepsia 1987; 28: 142-146.