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CLINICAL SIGNIFICANCE OF MEASUREMENT OF % FREE VALPROIC ACID IN EPILEPTIC CHILDREN

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The degree of protein binding of valproic acid (VPA) was determined in 127 epileptic patients ranging in age from 16 months to 17 years in order to evaluate the effects of combined therapy and total VPA levels on the % free VPA fraction, and the relationship between free VPA level and the occurrence of adverse effect of VPA in pediatric practice.

An approximately three-fold inter-individual variation in the % free VPA fraction was observed in both monotherapy and polytherapy groups. Combined therapy with other anti-epileptic drugs produced no noticeable effects on the % free VPA fraction. The mean % free VPA fraction in the subgroups with total VPA $\geqq 80$ μg/ml were much greater than those in the subgroups with total VPA level <80 μg/ml ($p<0.001$). The occurrence of adverse effects was related to neither the free nor total VPA levels.

Introduction

Valproic acid (VPA) has been used as a first-line anti-epileptic drug for the treatment of generalized and partial epilepsy. As a branched-chain fatty acid, VPA is mainly bound to plasma proteins in a concentration-dependent manner[1)~4)]. Thus, small changes in drug binding may significantly alter the free drug fraction. Some states such as uremia, nephrotic syndrome, hypoalbuminemia, liver dysfunction and concomitant administration of other drugs such as salicylate can lead to a decrease in protein binding of VPA and a corresponding increase in the free VPA level[5)~8)].

Because VPA is a fatty acid, it competes with free fatty acids for protein binding sites[9)10)]. In the presence of a high level of free fatty acids, the free VPA level will increase. As a result, significant diurnal fluctuation in VPA protein binding may be related to normal changes in levels of free fatty acids, which displace VPA from albumin[11)12)]. Fluctuation of the free drug levels may be twice as great as fluctuation of total levels[13)].

Some investigators have reported a decrease in VPA protein binding with an increase in the total VPA level[2)8)]. Clinically significant increases in the % free VPA fraction are first noted when the total VPA level exceeds 80 μg/ml, which may be related to saturation of drug-binding sites on plasma proteins.

In view of these findings, free VPA level monitoring would seem to be a beneficial guide to therapy. However, the clinical relevance of monitoring total versus free levels of VPA has not been adequately evaluated in clinical trials, especially in the pediatric population. This paper evaluates the effects of combined therapy and total VPA levels on the % free VPA fraction, and the relationship between the VPA level and occurrence of adverse effects in epileptic children.

Subjects and Methods

The subjects of this study were 127 epileptic patients of both sexes (68 male, 59 female), ranging in age from 16 months to 17 years. They were all followed as inpatients or outpatients at the Department of Pediatrics, Tokyo Women's Medical College Hospital. Of these 127 patients, 46 were treated with VPA as the sole anti-epileptic drug. The remaining 81 patients had been receiving VPA in combination with other anticonvulsants such as phenytoin (PHT), carbamazepine (CPZ), phenobarbital (PB), clonazepam (CZP) or acetazolamide (AZA). All of them had received regular VPA administration for at least 3 months. No attempt was made to bias the patient selection; however, some patients with clinical conditions such as nephrotic syndrome, hypoalbuminemia, hyperbilirubinemia, gastrointestinal diseases, and concomitant salicylate administration were excluded from this study.

Blood samples of 4 ml were collected within 2~4 hours after the morning dose. Each sample was immediately centrifuged and the serum was divided into two aliquots to measure the total and free levels, respectively. The % free fraction of the drug is the free drug level divided by the total drug level expressed as a percentage. The total serum level of VPA was determined using TDX Reagent Packs, Calibrators, and Controls (TDX; Abbott Lab, USA). The free VPA level analysis involved ultra filtration of the serum samples with EMIT Free Level filters (Syva Corp, Palo Alto, USA) by centrifuging at 2,000×g for 40 minutes in order to remove the protein. The ultra filtrates were the analyzed using TDX Free Reagent Packs, Calibrators and Controls. All procedures were performed at room temperature.

Statistical analysis was performed by means of Student's t test.

Results

Effect of combined therapy on % free VPA fraction:

The mean % free VPA fraction for the 127 patients in the study was 10.6% with a standard deviation (SD) of 2.72%. The mean ± SD for the 46 patients on monotherapy was 10.67 ± 2.63% (range, 5.5~18.8%) and for the 81 patients on polytherapy 10.56 ± 2.72% (range, 5.9~17.8%). There was no statistically significant difference between these two groups.

Effect of serum total VPA level on % free VPA fraction:

The two groups (monotherapy and polytherapy) were further subdivided into two subgroups according to total VPA level (≥80 μg/ml subgroup

Table 1 Effects of total VPA level on the % free VPA fraction

	Total VPA level (μg/ml)	No	Free VPA fraction (%)
VPA monotherapy	<80	30	9.16±2.18
	≥80	16	13.49±3.52*
Polytherapy	<80	63	9.47±2.45
	≥80	18	14.37±3.36*

*$p<0.001$ versus the subgroups with total VPA level<80 μg/ml.

Table 2 Total, free VPA levels, % free VPA fraction in 4 patients with adverse effects of VPA

Case	Sex	Age (y/m)	Clinical features	Abnormal lab tests	Total VPA level(μg/ml)	Free VPA level(μg/ml)	Free VPA fraction(%)	Other AED
1	F		nausea, vomiting, epigastralgia	amylase 3,200	82	9.73	11.9	CBZ
2	M	4/6	transient consciousness disturbance	ammonia 182	75	11.8	15.8	CBZ
3	M	8/10	asymptomatic	ammonia 156	94	11.3	12.2	CZP
4	M	3/7	asymptomatic	ammonia 140	84	7.16	9.1	

F: female, M: male y/m: year/month, Normal range of ammonia: 60-120μg/dl, Normal range of amylase: 135-360U/L.

and <80 μg/ml subgroup). It is apparent from Table 1 that the mean values of % free VPA fraction in the subgroups with total VPA ≥80 μg/ml were much greater than those in the subgroups with total VPA level <80 μg/ml in both the monotherapy and polytherapy groups ($p<0.001$).

Relationship between free VPA level and adverse effects of VPA:

Among these 127 patients receiving VPA, only four were thought to have experienced an adverse effect of VPA including 1 case of acute pancreatitis and 3 cases of hyperammonemia. Their clinical manifestations and VPA free and total levels are summarized in Table 2. On the other hand, there were 23 out of the 127 patients with free VPA levels exceeding 10 μg/ml (range, 10.5~21.6 μg/ml) and 11 patients with total VPA levels exceeding 100 μg/ml (range, 104~122 μg/ml) who were clinically asymptomatic and had normal laboratory tests. These results failed to demonstrate a good correlation between VPA levels (total and free) and the occurrence of adverse effects of VPA.

Discussion

Neither the free fraction nor the total drug level adequately reflects the amount of drug available to tissues, and only the free drug level is considered to be responsible for the pharmacologic effect and the adverse reactions. However, some anti-epileptic drugs such as ethosuximide and primidone exhibit practically no plasma binding. Measurement of free levels of such drugs is unnecessary. The criteria for consideration of monitoring of free drug level include: (1) the drug is known to exhibit highly variable protein binding, such as is the case with PHT, VPA and CBZ[14], (2) the patient's clinical status does not correlate with the therapeutic total drug level, (3) the patient is receiving multiple drugs with a potential for protein displacement interaction, for example VPA can displace PHT from plasma protein[15], and (4) the patient has a concomitant disease that affects protein binding such as uremia.

The fluctuation in VPA binding is much greater than that of PHT or CBZ, making VPA free levels more difficult to predict from total levels. However, there is no clinical evidence that therapeutic or adverse effects correlate highly with the free than total level of VPA.

An approximately 3-fold inter-individual variation in the % free VPA fraction was observed in this series. This indicates that the free VPA level does not necessarily depend on the total VPA level. The clinical findings suggest that the clinical adverse effects of VPA are not related to either the total or the free VPA level. The appropriate therapeutic ranges for total and free VPA levels remain poorly defined. In conclusion, there is still little evidence indicating the necessity for routine free VPA monitoring in the treatment of epileptic children. Regular evaluations including liver function, ammonia, and amylase are recommended in all patients receiving VPA therapy, irrespective of their serum VPA levels.

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小児てんかん患児における血中遊離バルプロ酸%分画測定の臨床的意義

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他の抗てんかん剤の併用，ならびに血中バルプロ酸総濃度が，血中遊離バルプロ酸%分画に及ぼす影響を評価し，また血中遊離バルプロ酸濃度とバルプロ酸の臨床的副作用との関係を検討する目的で，最低3カ月以上バルプロ酸を服薬中の小児てんかん患児（年齢：16カ月～17歳）127例について，血中総濃度，遊離分画濃度および%分画を測定し，バルプロ酸の血中蛋白結合状態を検討した．

その結果，全対象例の遊離バルプロ酸%分画平均値は10.6±2.7%であり，症例間の個人差は大きく，約3倍に達した．しかし単独療法群と併用療法群の間に有意差はなかった．また血中総濃度が80 μg/ml 以上群の血中遊離バルプロ酸%分画は，80μg/ml 未満群のそれに比し，有意に大であった（$p<0.001$）．臨床的副作用の発現と血中バルプロ酸総濃度あるいは%分画との間には，相関は認められなかった．