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Effect of chronic valproic acid treatment on plasma and urine carnitine levels in children: decreased urinary excretion

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Plasma levels and urinary carnitine excretion rates were determined in children treated with valproic acid (n = 11) and in age and sex matched controls (n = 11). Urine was collected throughout two consecutive 24 h periods in both groups, and blood samples were taken on the first day of collection after an overnight fast. The plasma level of total and free carnitine was significantly lower in the treated group (24.3 ± 2.2 vs 34.9 ± 2.4 and 16.8 ± 1.8 vs 26.5 ± 2.1 nanomol/ml; values are means ± SEM), while there was no significant alteration in the acylcarnitine fraction. In the treated group of children a significant reduction was found in the plasma beta-hydroxybutyrate level indicating a limited fatty acid utilization (23.2 ± ± 2.5 vs 81.9 ± 7.8 nanomol/ml). Urinary total and free carnitine decreased from 286.4 ± 57.8 to 120.8 ± 18.2 and from 154.3 ± 33.6 to 21.2 ± 5.8 μmol/day, respectively; the acyled fraction was not significantly reduced. In one child, urinary carnitine excretion was followed during the first ten days of treatment. After the 2nd day a decrease of the total and free fraction was observed, confirming previous data obtained during chronic VPA treatment. It has been concluded that the decreased plasma carnitine associated with chronic VPA treatment is not a result of an increased excretion rate, but more likely the consequence of a relatively insufficient endogenous carnitine synthesis. The decreased plasma BOB level probably due to limited fatty acid utilization might also be a metabolic consepuence of depressed carnitine concentration.

Valproate, 2-n-propylpentanoate, as an antiepileptic drug is widely used in clinical practice due to its effectiveness against a broad spectrum ofse izures [15, 23]. Despite of its popularity, exten sive interest has been focussed on its side effects [5, 12], inluding its teratogenicity [8] and the liver injury induced by it. The latter has been shown to involve microvesicular steatosis resembling the liver damage associated with Reye's syndrome and carnitine deficiency [2, 11, 27].

As to the pathomechanism of liver damage the role of secondary carnitine deficiency has been suggested by several authors [7, 20]. Valproic acid, being a branched chain fatty acid, may theoretically conjugate with car-

Abbreviations: VPA valproic acid; BOB beta-hydroxybutyrate

nitine via carnitine acyltransferases [1], as it occurs in the case of other medium and long chain fatty acids [4, 13, 22]. It appeared resonable to suggest that the elevated excretion of valproylcarnitine and/or its derivatives may produce an increased urinary acylcarnitine loss resulting in secondary carnitine deficiency. The purpose of the present study was to assess this hypothesis.

Patients and Methods

Eleven handicapped children subjected to VPA monotherapy were selected for the study. Clinical diagnoses included infantile spasm, generalized convulsions, Lennox–Gastaut syndrome and Jackson's focal convulsions. The duration of treatment ranged from 3 to 36 months, the drug was administered in two or three doses daily. Eleven age and sex matched healthy patients served as control subjects. They were hospitalized for observation but no abnormalities were detected. Informed consent was obtained from the parents of all participants in the study.

All patients were fed the same clinical diet. After a regular supper the children were requested to fast overnight. On the next day a 48 h continuous urine collection was started between 7.00 and 8.00 A.M. Each fraction was frozen in plastic containers separately in the two 24 h periods. Venous blood samples were taken on an empty stomach on the first day of urine collection into test tubes containing heparin and centrifuged immediately. The resulting plasma and the collected urine were stored at −20 °C until analysis.

SGOT and SGPT were determined using assay kits purchased from Boehringer (Mannheim, F.R.G.)

Carnitine was quantitated by the radiochemical method of Cederblad and Linstedt [3] with some modifications as described previously [14]. BOB was determined in the neutralized perchloric acid extract of plasma by standard enzymatic analysis [26]. Statistical significance of the results was calculated by Student's t test.

Results

The mean $\pm$ SEM values of plasma and urine total, free and acylcarnitines observed in the two groups of children are shown in Table I. There was a marked depression in the plasma total and free carnitine levels ($p < 0.005$); the decrease of the acylated fraction was not significant compared to the control group. Plasma BOB concentration was significantly lower in the treated children than in the controls (23.2 ± 2.5 vs 81.9 ± 7.8 nanomol/ml; $p < 0.05$). In both groups SGOT and SGPT activities were within the normal range (not shown).

Quantities of excreted carnitine are also shown in Table I. The daily amount of urinary total and free carnitine was found to be significantly lower in VPA treated patients than in controls ($p < 0.02$ and $p < 0.001$, respectively). The difference was more pronounced in the free portion whereas its excreted amount was approximately 14% of the control value. The decrease in the acylated fraction was not significant statistically.

Urinary total and free carnitine excretion was followed during the first 10 days of treatment in a child suffering from infantile spasm. The total and free urinary carnitine values

TABLE I

Comparison of plasma carnitine levels and urinary carnitine extraction in VPA treated and control children. (Values of urinary carnitine excretion are means of two consecutive 24 h collection period. See text)

Patient	VPA dose (mg/day)	age-sex	Serum carnitine (nmoles/ml)			Urine carnitine (μmoles/day)		
			total	free	acyl	total	free	acyl
1	600	$6^{1}/_{12}$ m	23.4	10.4	13.0	71.9	16.3	55.6
2	900	$6^{5}/_{12}$ m	30.9	14.6	16.3	68.5	4.7	63.8
3	600	$8^{10}/_{12}$ m	21.8	16.0	5.8	259.2	52.1	207.1
4	600	$9\ ^{1}/_{6}$ f	12.6	9.2	3.4	87.2	3.7	83.5
5	600	$9\ ^{7}/_{12}$ m	18.3	14.2	4.1	110.3	15.3	95.0
6	900	$9^{10}/_{12}$ f	32.1	22.8	9.3	118.1	30.1	88.0
7	600	$10\ ^{8}/_{12}$ m	29.3	23.8	15.5	60.5	4.3	56.2
8	1200	$13\ ^{8}/_{12}$ f	24.9	20.8	4.1	108.5	24.2	84.3
9	1200	$14\ ^{4}/_{12}$ f	14.3	11.2	3.1	204.0	60.6	143.4
10	1200	$14^{10}/_{12}$ f	24.2	15.4	8.8	131.4	13.8	117.6
11	1200	$15^{10}/_{12}$ m	35.4	26.7	8.7	109.6	7.6	102.0
	mean ± SEM		24.3±2.2	16.8±1.8	7.5±1.3	120.8±18.2	21.2±5.8	99.7±13.3
12	—	$6\ ^{3}/_{12}$ m	40.5	35.3	5.2	204.1	108.0	96.1
13	—	$6\ ^{1}/_{12}$ m	30.7	20.8	9.9	151.2	64.6	86.6
14	—	$8\ ^{8}/_{12}$ m	33.1	20.7	12.4	212.7	134.7	78.0
15	—	$9\ ^{5}/_{12}$ f	52.4	39.9	12.5	701.7	435.4	266.3
16	—	$9\ ^{7}/_{12}$ m	31.8	28.0	3.8	304.7	144.4	160.3
17	—	$9^{10}/_{12}$ f	36.6	30.6	6.0	280.8	174.4	106.4
18	—	$10\ ^{6}/_{12}$ m	22.3	17.5	4.8	590.9	261.7	329.2
19	—	$13\ ^{1}/_{12}$ f	30.5	23.7	6.8	280.0	140.7	139.3
20	—	$14\ ^{1}/_{12}$ f	39.8	25.2	14.6	160.8	119.9	40.9
21	—	$14^{11}/_{12}$ f	28.3	19.2	9.1	198.0	91.4	106.6
22	—	$15\ ^{8}/_{12}$ m	37.9	30.9	7.0	65.0	22.1	42.9
	mean ± SEM		34.9±2.4	26.5±2.1	8.4±1.1	286.4±57.8	154.3±33.6	132.1±7.22

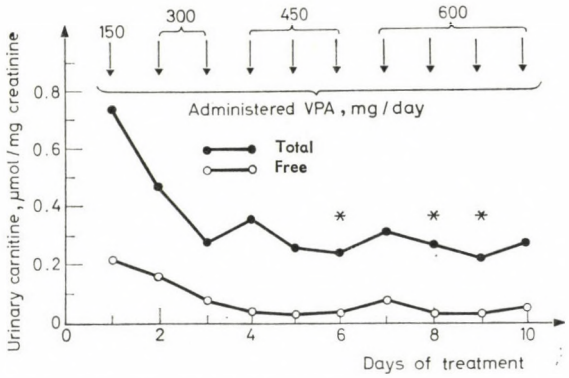

FIG. 1. Urinary carnitine excretion in a child with infantile spasm during the first 10 days of VPA administration. (On the days marked with asterisks enuresis occurred in connection with seizures.)

are shown in Figure 1. Unfortunately on days 6, 8, and 9 the collection of urine was not complete, because of enuresis associated with seizures. In the 5 years old child administration of VPA resulted in a fall of total as well as free carnitine excretion. Plasma values of total and free carnitine obtained on the 9th day of VPA administration were also lower than before the treatment (52.0 vs 23.4 and 44.7 vs 10.4 nanomol/ml, respectively).

Discussion

An important factor in VPA associated toxicity could be an impairment of liver metabolism similar to that found in Reye's syndrome and carnitine deficiency. Although therapeutic doses of VPA are normally well tolerated, impairment of carnitine metabolism has been suggested by several investigators [7, 9, 20]. Ohtani et al [20] found that patients taking VPA had lower carnitine levels in plasma. In VPA induced Reye syndrome Böhles et al [2] observed a reduced serum carnitine level. According to the current view carnitine deficiency exists when there is insufficient carnitine to buffer toxic acyl compounds [24]. The significantly lower plasma total and free carnitine levels observed in VPA treated children also pointed toward the impairment of carnitine metabolism.

The primary function of carnitine is to transfer long chain fatty acids across the inner mitochondrial membrane to the matrix space [10]. Since carnitine and the related enzymes have a key role in hepatic regulation of fatty acid oxidation and ketogenesis [16]; utilization of fatty acids is limited in a wide spectrum of primary and secondary carnitine deficiency syndromes [9]. Several observations suggest that VPA may interfere with fatty acid utilization by a complex mechanism [6, 18, 19, 21] leading to decreased ketogenesis [25]. The re-

duced fasting ketonaemia found in VPA treated children may be, in part, a metabolic consequence of the observed relative carnitine deficiency.

The biochemical background of depressed carnitine levels demonstrated in VPA treated subjects is unclear. It might result from excessive loss of carnitine due to an increased urinary excretion [20]. Recently Millington et al [17] have reported an augmented total and acylcarnitine levels in random urine samples of three children in different stages of chronic VPA therapy, suggesting an enhanced excretion. In contrast, the significantly reduced total and free carnitine excretion and the lower plasma carnitine levels obtained in the present study may be explained rather by insufficient endogenous carnitine synthesis.

The onset and mechanism by which VPA affects carnitine metabolism is not known. Still, the marked fall in carnitine excretion observed in one patient after the second day of treatment suggests that the response of carnitine metabolism may be an early and useful indicator of the adverse effects of VPA medication. This association may reflect impaired nutrition. Children with mental retardation of those with severe and frequently recurring seizures are often difficult to feed and may become malnourished. In the presence of reduced food intake reduced exogenous carnitine intake may occur [25], raising the issue that the child monitored in the present study was already carnitine deficient.

Regarding the prevention of the adverse effects of VPA, many recommendations have been made (7, 25). In patients taking the drug Ohtani et al (20) found that serum carnitine levels returned to normal and serum ammonia levels fell in response to carnitine supplementation. Coulter recommended carnitine rich natural foods for prevention (7). In agreement with others (7, 20, 24) we think that further controlled trials of carnitine supplementation are needed in patients at risk treated with VPA, to show whether the liver injury can be prevented or reversed by carnitine administration.

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