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The effect of carnitine supplementation in valproate-induced hyperammonaemia

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The aim of the study was to investigate the effect of carnitine supplementation in valproic acid (VPA) treated patients presenting with increased plasma ammonia concentrations. Plasma ammonia concentrations were recorded in 69 children and young adults on VPA monotherapy (25.6 ± 9.2 mg VPA/kg per day; mean plasma VPA concentration 68.8 ± 27.6 mg/l). Their mean plasma ammonia concentration was 80.2 ± 32.1 μg/dl (median 73.1 μg/dl). A total of 24 patients (35.3%) presenting with ammonia concentrations >80 μg/dl were considered hyperammonaemic. Of these, 15/24 (22.1%) showed ammonia concentrations >100 μg/dl, even up to 194 μg/dl. In 48/69 patients, plasma carnitine concentrations could be determined. The plasma total carnitine (TC) concentrations were rather low (26.9 ± 8.8 μmol/l) compared to normal values obtained in our laboratory (40.9 ± 7.2 μmol/l). The percentage of free carnitine was considered decreased (<75% TC) in 13/48 samples (27%). Fourteen hyperammonaemic patients and one with a plasma ammonia level of 60 μg/dl agreed to be supplemented with L-carnitine (1 g/m^2 per day divided into two equal doses). Their plasma ammonia and carnitine concentrations were re-evaluated after a mean of 9.1 ± 4.0 days (median 9.0 days) and in 9 patients again after a mean of 79.6 ± 30.1 days (median 75 days) of L-carnitine supplementation. Plasma ammonia concentrations decreased in all 15 patients. The decrease was 25.4 ± 11.2% (median 28.3%) after a mean of 9.1 ± 4.0 days and amounted to 46.0 ± 17.2% (median 48%) after 79.6 ± 30.1 days. L-Carnitine supplementation led to an increase in plasma free carnitine of 11.6 ± 13.0% (median 15.6%) and to a further increase of 11.1 ± 8.4% (median 11.5%) when re-evaluated a second time. The plasma ammonia concentrations were significantly correlated with the percentage of free plasma carnitine ($r = -0.67$; $p < 0.0001$). The results show that carnitine supplementation is a means of normalizing elevated plasma ammonia concentrations. However, we cannot conclude from our results whether this lowers the risk of developing a VPA-induced Reye's-like syndrome. □ *Carnitine, carnitine insufficiency, carnitine supplementation, hyperammonaemia, valproate*

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Valproic acid (VPA), dipropyl acetic acid, has become a widely used drug for the treatment of epilepsy. Since 1979, metabolic side-effects of VPA therapy, such as asymptomatic hyperammonaemia (1) and even acute hepatocerebral dysfunction (Reye-like syndrome), have been described, and low plasma carnitine levels have been reported (2–5). A decreased availability of free carnitine may play a role in the aetiology of the VPA-induced hepatopathy. This study was designed to investigate the effect of carnitine supplementation in VPA-treated patients presenting with increased plasma ammonia concentrations.

Subjects and methods

Subjects

Plasma ammonia concentrations were recorded in 69 children and young adults (35 M: 34 F) on VPA monotherapy (25.6 ± 9.2 mg VPA/kg per day) attending our neuropediatric outpatient clinic. Blood was taken about 3 h after breakfast and VPA intake. The VPA intake led to an average serum VPA concentration of 68.8 ± 27.6 mg/l. The patients were aged 1–24 years (median 9.5 years). In 48/69 patients plasma carnitine concentrations could be determined. Fourteen patients (10 M, 4 F; age range 1–19 years, median age 6 years) with plasma ammonia concentrations >80 μg/dl and one patient with a plasma ammonia level of 60 μg/dl (Table 1) agreed to be supplemented with L-carnitine (1 g/m^2 per day divided into two equal doses). The plasma ammonia and carnitine concentrations were re-evaluated in 9 patients after a mean of 9.1 ± 4.0 days (median 9.0 days) and in 10 patients again after a mean of 79.6 ± 30.1 days (median 75 days) of L-carnitine supplementation. Blood was drawn between 10 and 11 a.m., which was about 3–4 h after carnitine and VPA intake. The experimental purpose of the carnitine supplementation has been explained to the

Table 1. Characterization of 15 epileptic children on carnitine supplementation. [a]Classified according to the International League against Epilepsy (ILAE).

Sex	Age (years)	Duration of VPA therapy (years)	VPA dosage (mg/kg per day)	VPA concentration (mg/l)	Seizure type[a]
M	3	1	31.5	41.1	Myoclonic
M	5	2	30.0	56.5	Tonic-clonic
M	4	2	50.0	104.1	Tonic-clonic
M	11	4	21.7	42.6	Tonic-clonic
M	5	3	22.2	72.0	Tonic-clonic; Fanconi syndrome
F	13	4	31.9	40.2	Tonic-clonic
M	12	4	34.3	87.0	Tonic-clonic
M	17	2	25.0	47.5	Tonic-clonic
M	8	3	35.0	45.3	Tonic-clonic
M	6	3	25.0	79.6	Tonic-clonic
M	17	4	23.0	69.6	Tonic-clonic
M	16	5	7.2	39.2	Absent with tonic component
F	1	$\frac{3}{12}$	30.0	37.8	Tonic-clonic
F	1.5	$\frac{7}{12}$	32.0	76.0	Myoclonic
F	6	2	30.0	49.7	Tonic-clonic

[a]Classified according to the International League against Epilepsy (ILAE).

patients and their parents. All had agreed to being included in the study.

Methods

Plasma ammonia was determined using the Monotest (Boeringer-Mannheim). Carnitine levels were measured according to McGarry and Foster (6), with modifications as described elsewhere (7).

Normal plasma carnitine concentrations (determined in our laboratory) are: total carnitine 40.9 ± 7.2 μmol/l; free carnitine 36.05 ± 6.1 μmol/l; and acylcarnitine 5.6 ± 2.6 μmol/l.

Statistical analysis of the data was performed using the StatView 512+ program to undertake a paired *t*-test and linear regression analysis.

Results

At the beginning of the investigation the 69 patients on VPA therapy presented an average serum VPA

Table 2. Plasma concentrations of NH_3, total carnitine, per cent free carnitine and the acyl carnitine/free carnitine (AC/FC) ratio in epileptic children treated with valproic acid monotherapy.[a]

	Before carnitine		After carnitine supplementation		
			9.1 ± 4 days		79.6 ± 30.1 days
Plasma NH_3[b] (μg/dl)*	116.3 ± 39.2	$p < 0.0004$	80.1 ± 27.6	NS	67.2 ± 21.4
			$p < 0.001$		
Total carnitine (μmol/l)	23.7 ± 6.3	$p < 0.0005$	37.0 ± 7.0	NS	36.0 ± 19.2
			NS		
Free carnitine (%)	58.6 ± 16.1	$p < 0.05$	69.1 ± 7.2	NS	71.0 ± 13.6
			$p < 0.02$		
AC/FC	0.74 ± 0.50	$p < 0.01$	0.43 ± 0.10	NS	0.46 ± 0.38
			$p < 0.05$		

[a]The children were investigated before ($n = 15$) supplementation with L-carnitine (1 g/m^2 per day) and after 9.1 ± 4.0 ($n = 9$) and 79.6 ± 30.1 days ($n = 10$).

[b]In 4.3 ± 2.4 presentations before this intervention study the plasma ammonia concentrations were 112 ± 42 μg/dl.

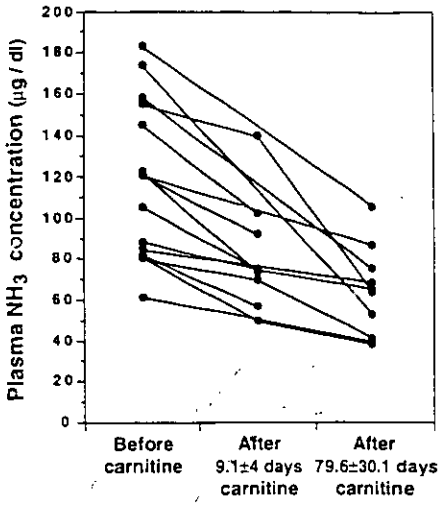

Fig. 1. Decreasing plasma ammonia concentrations in VPA-treated patients ($n = 15$) after supplementation with L-carnitine (1 g/m^2 per day).

concentration of 68.8 ± 27.6 mg/l. Their mean plasma ammonia concentration was 80.2 ± 32.1 µg/dl (median 73.1 µg/dl). Twenty-four of the 69 patients (16 M; 8 F) (35.3%) were considered hyperammonaemic, presenting plasma ammonia concentrations of >80 µg/dl. Fifteen of these 69 (22.1%) showed ammonia concentrations of >100 µg/dl and even up to 194 µg/dl.

The concentrations of plasma ammonia, total carnitine, the percentage of free carnitine and the acyl carnitine/free carnitine ratio (AC/FC) in the L-carnitine supplemented patients ($n = 15$) are presented in Table 2. Plasma ammonia concentrations decreased in all 15 patients (Fig. 1). The decrease was 25.4 ± 11.2% (median 28.3%) after a mean of 9.1 ± 4.0 days of L-carnitine supplementation and amounted to 46.0 ± 17.2% (median 48%) after 79.6 ± 30.1 days. The plasma total (TC) and free carnitine (FC) concentrations, as determined in 48 patients, were rather low

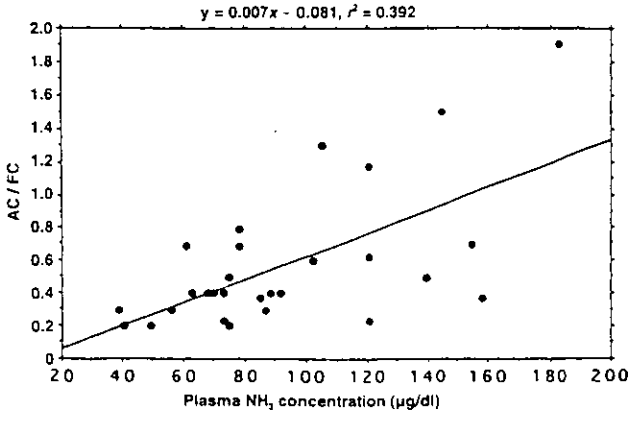

Fig. 2. Correlation of the plasma ammonia concentrations with the AC/FC ratio ($r = -0.67$; $p < 0.0001$).

(TC 26.9 ± 8.8 µmol/l; FC 17.4 ± 5.4 µmol/l) compared with the normal values (TC 40.9 ± 7.2 µmol/l; FC 36.05 ± 6.1 µmol/l). Free carnitine was decreased to <75% of total carnitine in 13/48 samples (27%). One 5-year-old boy presenting with an extremely decreased total carnitine concentration of 10.8 µmol/l was diagnosed as suffering from Fanconi syndrome.

L-Carnitine supplementation led to a 11.6 ± 13% (median 15.6%) increase in plasma free carnitine within 9.1 ± 4.0 days of treatment and to a further increase of 11.1 ± 8.4% (median 11.5%) after 79.6 ± 30.1 days. This increase in free carnitine concentrations was also reflected in a relative normalization of the AC/FC ratio. The plasma ammonia concentrations were significantly correlated with the percentage of free carnitine ($r = -0.67$; $p < 0.0001$) (Fig. 2) as well as with the AC/FC ratio ($r = 0.62$; $p < 0.0004$). No correlations of these data were found with age, sex, dosage or plasma VPA concentration.

Discussion

Asymptomatic hyperammonaemia is frequently reported to be associated with VPA therapy (5, 8–11). There is still much controversy about the pathophysiological basis of this hyperammonaemia. Interference with urea synthesis has been discussed (12) as has altered renal glutamine metabolism (13–15). Hyperammonaemia, as defined by a plasma ammonia concentration of >80 µg/dl, was shown by about 35% of our patients, which is in agreement with the generally reported high frequency of this disturbance (3, 10, 16). The present results confirm those studies reporting no relationship between plasma ammonia concentrations and age, time on VPA therapy, VPA dosage and plasma VPA concentration (3, 16). Nevertheless, two studies have shown a correlation between plasma ammonia concentration and VPA intake (5). The reduced plasma free carnitine concentrations found in 27% of our patients add to the amount of existing data emphasizing a reduction in plasma free carnitine concentrations (3, 4, 9, 10, 14, 17–26). The incidence is reported to be up to 76.5% in patients on VPA. The reduced plasma carnitine concentrations are indicative of a secondary carnitine insufficiency. According to the present understanding, this may be a consequence of valproylcarnitine formation and its ensuing excretion into the urine as well as of a VPA-induced inhibition of cellular carnitine uptake (27). An even more complex pathophysiological mechanism has been suggested as the cause of carnitine insufficiency by Rozas et al. (28). The coefficient of determination (r^2) of the correlations between the plasma concentrations of ammonia and free carnitine, however, reveal that decreased plasma carnitine concentrations explain about 40–45% of observed hyperammonaemia. The remainder may be explained by a renal pathophysiological component,

which hitherto has only been suggested on the basis of animal studies (13–15). We confirm those studies, showing carnitine administration to be protective against asymptomatic hyperammonaemia (8, 9) and against the alteration in carnitine metabolism induced by the administration of VPA (8, 29). As our patients presented hyperammonaemia after months or years of VPA therapy, we cannot support the opinion presented by Laub (30) that hyperammonaemia is only a transient phenomenon that will normalize even without carnitine supplementation. Our correlation between plasma free carnitine and the plasma ammonia concentration is almost identical to that presented by Ohtani et al. (5). A normalization of the plasma ammonia concentration was reported by these authors after 4 weeks of carnitine administration, which is comparable to the time courses observed in our patients.

Our results substantiate the recommendation that carnitine supplementation should be considered in VPA-treated patients when plasma ammonia concentrations are inappropriately increased or when the plasma AC/FC ratio is increased above 0.4 in the fed patient. The first ammonia and carnitine determinations should be performed 2 weeks after the therapeutic VPA level has been attained. The necessary carnitine dosage can be titrated, keeping the AC/FC ratio within the normal range. However, we cannot conclude from our results whether or not the prevention of VPA-induced hyperammonaemia lowers the risk of developing a VPA-induced Reye's-like syndrome.

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