L-carnitine enhances excretion of propionyl coenzyme A as propionylcarnitine in propionic acidemia.

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Research Article

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Rapid Publication

L-Carnitine Enhances Excretion of Propionyl *Coenzyme* A As Propionylcarnitine in Propionic Acidemia

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Abstract. Treatment with L-carnitine greatly enhanced the formation and excretion of short-chain acylcarnitines in three patients with propionic acidemia and in three normal controls. The use of fast atom bombardment mass spectrometry and linked scanning at constant magnetic (B) to electric (E) field ratio identified the acylcarnitine as propionylcarnitine in patients with propionic acidemia. The normal children excreted mostly acetylcarnitine. Propionic acidemia and other organic acidurias are characterized by the intramitochondrial accumulation of short-chain acyl-Coenzyme A (CoA) compounds. The substrate specificity of the carnitine acetyltransferase enzyme and its steady state nature appears to facilitate elimination of propionyl groups while restoring the acyl-CoA:free CoA ratio in the mitochondrion. We suggest that L-carnitine may be a useful therapeutic approach for elimination of toxic acyl CoA compounds in several of these disorders.

Introduction

In a previous study (1), we reported a beneficial clinical response in a patient with propionic acidemia due to deficient propionyl-Coenzyme A (CoA)[1] carboxylase following a single dose of L-carnitine (25 mg/kg). This response was associated with a rapid increase in urinary hippurate excretion. Hippurate synthesis is an intramitochondrial function that requires adequate amounts of ATP and free Coenzyme A (CoASH). Therefore, it was suggested that cofactors were made more available by giving carnitine to this patient, probably due to acylcarnitine formation, which thereby restored intramitochondrial CoASH concentration more toward normal. This study, including the previous patient (1), presents the enhanced excretion of short-chain acylcarnitines after administration of L-carnitine to three patients with propionic acidemia and to three normal controls. The identification of the specific excreted acylcarnitines is also described.

Methods

Serum and urinary carnitine and acylcarnitines were determined by the radioenzymatic assay utilizing [^{14}C-1]acetyl-CoA and carnitine acetyltransferase as described by Brass and Hoppel (2). In this method, serum acylcarnitines are separated into an acid-soluble fraction that corresponds to short-chain acylcarnitines and into an acid-insoluble fraction containing long-chain acylcarnitines. The results are expressed in micromole per liter (μM) for serum or as nanomole per milligram creatinine for urine.

Acylcarnitines (acetyl, propionyl, isobutyryl, butyryl, iso-valeryl, hexanoyl, octanoyl, and decanoyl) were synthesized according to the method of Bohmer and Bremer (3) using the acylchlorides and L-carnitine under reflux conditions and were purified by subsequent multiple recrystallizations. Urine samples from the patients were passed over Dowex-1 columns at neutrality (4) and the aqueous eluates containing acyl-

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1. *Abbreviations used in this paper:* B/E linked scan, magnetic (B) to electric (E) field ratio; CoA, Coenzyme A; CoASH, free Coenzyme A; FAB, fast atom bombardment; m/z, mass over charge.

carnitines were concentrated by lyophilization. These fractions and the synthesized acylcarnitines were analyzed on a VG-Analytical model 7070 HS spectrometer (VG-Analytical Ltd., Manchester, England) fitted with a fast atom bombardment (FAB) ion source/sample introduction system (5) and a linked-scanning facility at constant magnetic (B) to electric (E) field ratio (B/E linked scan) (6). Samples were applied from aqueous solution onto a stainless steel target that was coated with glycerol and were maintained in the ion source at a potential of +4 kV. FAB spectra were generated by bombardment of the target with xenon atoms. Intense, stable, and prolonged spectra were obtained from microgram amounts of acylcarnitines. The linked scan spectra were recorded without the use of collision gas.

Results

Three male patients (aged 7 mo, 3 yr, and 6 yr) who had isolated deficiency of propionyl-CoA carboxylase were studied after informed consent. Three other children, aged 4, 11, and 13 yr, were used as controls 1 yr after total recovery from Reye's Syndrome. The patients and controls were under good metabolic control at the time of the study as evidenced by normal plasma ammonia, glucose, electrolyte, and acid-base status. Initially, the patients received 100 mg/kg L-carnitine (Sigma Tau, Rome, Italy) orally in a single dose. They were subsequently maintained on L-carnitine, 25 mg/kg, four times daily, which was the usual dose for systemic carnitine deficiency, without complications for periods ranging from 6–18 mo.

Before L-carnitine was given (Table I), the mean plasma level of free carnitine from the propionic acidemia patients was 9.1 μM (normal 36.7±7.6 μM, mean±SD). After 48 h of supplementation, the plasma free carnitine increased to a mean value of 45.4 μM. The short-chain acylcarnitines were elevated (mean 26.0 μM, normal 5.7±3.5) before carnitine administration and increased to a mean value of 48.1 μM after carnitine was given. The plasma free carnitine level from the control patients increased from 32.3 μM before carnitine to 64.0 μM afterwards. The short-chain acylcarnitine fraction before carnitine administration averaged 3.5 μM and increased to 41.8 μM.

Table I. Plasma Carnitine Profile Before and After Carnitine Administration*

		Free carnitine	Acylcarnitines
Propionic acidemia			
Pre-carnitine	1	6.9	30.9
	2	8.6	22.4
	3	11.7	24.6
Post-carnitine	1	20.6	58.3
	2	41.7	44.0
	3	74.0	42.0
Controls			
Pre-carnitine	1	32.4	3.8
	2	33.3	2.6
	3	31.2	4.2
Post-carnitine	1	50.0	11.2
	2	85.0	57.0
	3	64.0	26.5

* Nanomole per milliliter plasma.

Table II. Urinary Carnitine Profile Before and After Carnitine Administration*

		Free carnitine	Acylcarnitines
Propionic acidemia			
Pre-carnitine	1	6.1	178.7
	2	48.2	278.4
	3	2.1	65.9
Post-carnitine	1	1,429.1	3,486.5
	2	469.7	2,589.4
	3	778.0	2,183.0
Controls			
Pre-carnitine	1	33.9	160.1
	2	13.0	61.0
	3	24.0	89.0
Post-carnitine	1	4,516.6	483.4
	2	6,576.0	3,069.0
	3	8,252.0	2,835.0

* Nanomole per milligram creatinine.

After administration of L-carnitine all patients revealed markedly enhanced excretion of both free carnitine and acylcarnitines (Table II). Free carnitine excretion was lower in the patients than in the controls; however, the levels of acylcarnitine excretion were comparable between the two groups.

The synthetic acylcarnitines were examined by FAB mass spectrometry. They exhibited intense parent cations of the type $(CH_3)_3N^+CH_2CH(OR)CH_2CO_2H$ (R equals acetyl, propionyl, etc.), as well as prominent fragment ions at mass over charge (m/z) 58, 85, 100, and 144 (7). The characteristic parent ion for propionylcarnitine has an m/z value of 218 while that for acetylcarnitine had an m/z value of 204. An ion at mass 218 was highly prominent and persistent in the FAB spectra based on the partially purified urine samples obtained after carnitine administration in patients with propionic acidemia. One of these spectra is reproduced in Fig. 1 *a*. The other aforementioned ions characteristic of acylcarnitines were also prominent in the spectrum, inter alia.

Accurate mass measurement of the m/z 218 ion observed in the patients was carried out by peak matching at a resolution of 6,000 (10% valley); a glycerol matrix ion (m/z 185.1025) was used for reference. The m/z 218 ion seen in the urine samples gave a value of 218.1396, which is consistent with the formula

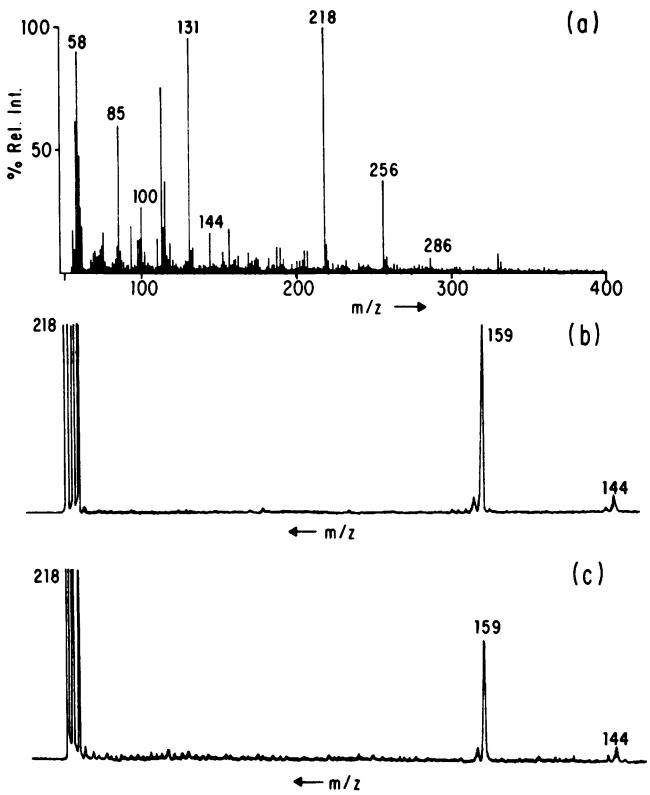

Figure 1. (*a*) FAB mass spectrum of postcarnitine urine purified over Dowex 1 resin. Ions at m/z 218, 144, 100, 85, and 58 are characteristic of propionylcarnitine. (*b*) Constant B/E linked scan spectrum from parent ion (m/z 218) of synthetic propionylcarnitine. (*c*) Constant B/E linked scan spectrum from m/z 218 ion in urine sample shown in *a*.

$C_{10}H_{20}O_4N$ expected for propionylcarnitine—calculated mass 218.1392. More compelling evidence for the presence of propionylcarnitine in these urines was obtained by linked scanning at constant B/E ratio, which provides an independent fragmentation pattern for each major ion in a mass spectrum (6). The constant B/E linked scan spectra for the m/z 218 ion from synthetic propionylcarnitine and the urine extract are compared in Fig. 1, *b* and *c*, respectively. The loss of 59 D from the parent ion appears to be a consistent and characteristic feature in the B/E linked scan spectra of all the acylcarnitines, and probably represents loss of the $(CH_3)_3N$ fragment. Therefore, the m/z 159 represents the remaining molecular fragment that is unique to propionylcarnitine (7). The ion at m/z 256 in Fig. 1 *a* has been identified as the potassium salt of propionylcarnitine.

No ion at mass 218 was observed in the urine of the control patients; instead, an ion of mass 204 predominated. This ion was analyzed by the techniques described above and elsewhere (7, 8) and was unequivocally identified as acetylcarnitine.

The patients with propionic acidemia have been maintained on oral carnitine supplement (25 mg/kg per 6 h) from 6–18 mo without complications. During this interval, the patients have not required bicarbonate for correction of metabolic imbalance. Intravenous glucose alone has been effective. Preliminary observations include patients' rapid improvement in fine and gross motor skills, increased interaction with their environment, and their improved expressive language. These changes have been noted within 1–2 wk of carnitine supplementation.

Discussion

Patients with isolated propionyl-CoA carboxylase deficiency had low serum-free carnitine (>2 SD below the mean) and had increased levels of short-chain acylcarnitines. In the urine, short-chain acylcarnitines were also markedly elevated relative to free carnitine. The total plasma carnitine levels were in the normal range, suggesting that carnitine biosynthesis is probably not impaired.

Low serum-free carnitine is often associated with organic acidurias (9–11), and an increased ratio of acylcarnitine to free carnitine has been observed at least in propionic acidemia, methylmalonic aciduria (8), and in a case of defective acyl CoA dehydrogenation (11). It seems likely that the abnormally high ratio of esterified to free carnitine in these conditions is due to accumulation of short-chain acyl CoA compounds, which are potential substrates for the carnitine acyltransferases.

When exogenous carnitine was given, there was an impressive response in the patients and controls as evidenced by increases in both serum-free carnitine and short-chain acylcarnitines. Similarly, there was an extraordinarily increased excretion of short-chain acylcarnitines in the urine. However, mostly acetylcarnitine was observed in the controls, while propionylcarnitine was the dominant species accounting for the urinary acylcarnitines in propionic acidemia. The unequivocal demonstration of propionylcarnitine as the major constituent of urinary acylcarnitines after treatment was accomplished by FAB mass spectrometry combined with B/E linked scanning.

The demonstration of enhanced production and excretion of propionyl groups as propionylcarnitine is an important and potentially useful therapeutic strategy. Elimination of propionyl groups as propionylcarnitine has also been demonstrated in methylmalonic aciduria (8). The in vivo synthesis of propionylcarnitine is almost certainly due to the action of carnitine acetyltransferase. This enzyme is located in mitochondria, peroxisomes, and in the endoplasmic reticulum, and, despite its name, its preferred substrate is propionyl-CoA rather than acetyl-CoA (12). Further, it is a steady state enzyme system and reflects the metabolic state of the individual (13). It would, therefore, have an important role in regulating the intramitochondrial propionyl-CoA/CoASH ratio in this disease by eliminating propionyl groups while increasing the availability of CoASH. Removal of propionyl-CoA would then lessen its well-known toxic effects as well as those of free propionate and of the metabolic product methylcitrate. These effects include inhibition of pyruvate dehydrogenase (14), *N*-acetylglutamate synthetase (15), the glycine cleavage system (16), succinate: CoA ligase guanosine diphosphate, and oxidative phosphorylation (17).

Increased intracellular diversion of propionyl-CoA to propionylcarnitine and its subsequent renal elimination would be expected to produce some clinical benefit. In our patients, these benefits included distinct and rapid benefit in fine and gross motor skills, improved expressive language, increased interaction with their environment, and elimination of the use of bicarbonate for metabolic correction, since intravenous glucose is effective. These preliminary observations suggest that supplementary carnitine is increasing muscle strength in these patients. Any impact on developmental skills, such as language, deserves further evaluation.

In both methylmalonic aciduria (8) and propionic acidemia, the excretion of propionyl groups as propionylcarnitine is augmented by carnitine treatment. The carnitine acyltransferases, then, provide an alternate pathway for elimination of this toxic organic anion. This newly recognized role for L-carnitine may have application in other organic acidurias in which the toxic compounds are also substrates for the carnitine acyltransferases.

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