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Effect of Carnitine Administration on Glycine Metabolism in Patients with Isovaleric Acidemia: Significance of Acetylcarnitine Determination to Estimate the Proper Carnitine Dose

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ITOH, T., ITO, T., OHBA, S., SUGIYAMA, N., MIZUGUCHI, K., YAMAGUCHI, S. and KIDOUCHI, K. *Effect of Carnitine Administration on Glycine Metabolism in Patients with Isovaleric Acidemia: Significance of Acetylcarnitine Determination to Estimate the Proper Carnitine Dose.* Tohoku J. Exp. Med., 1996, **179** (2), 101-109 —— In isovaleric acidemia (IVA), accumulated isovaleryl-CoA in the mitochondrion induces variable metabolic disturbances. To remove intramitochondrial isovaleryl groups, glycine therapy has been advocated primarily. On the other hand, secondary carnitine deficiency has been documented in this disorder and carnitine supplementation alone has been reported to be effective. In the present study, we administered carnitine and glycine to patients with IVA, and investigated serum carnitine and urinary excretion of total and free carnitine, acylcarnitine profile (i.e., isovalerylcarnitine and acetylcarnitine), and isovalerylglycine. By adding carnitine to glycine supplementation, more isovalerylglycine, not only isovalerylcarnitine, was excreted in the urine. Acetylcarnitine was detected in the urine only when sufficient carnitine was supplemented. We concluded that combined therapy of glycine and carnitine is more effective and safer to eliminate isovaleryl-CoA in IVA than conventional therapy using either glycine or carnitine. Urinary acetylcarnitine concentration might be a good marker indicating the optimal dose of L-carnitine supplementation. ———— isovalerylglycine; isovaleryl-CoA; isovalerylcarnitine; acetylcarnitine

Isovaleric acidemia (IVA) is one of inborn errors of leucine metabolism and it was first described in 1966 by Tanaka et al. The patients with IVA have

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defective activity of isovaleryl-CoA dehydrogenase and excrete large amount of isovalerylglycine (Tanaka and Isselbacher 1967) and other isovaleryl compounds into the urine.

In those cases, both glycine and L-carnitine have a possibility to detoxify isovaleryl compounds accumulated in tissues. But little is known about an influence of L-carnitine supplementation on glycine metabolism. We investigated here the bioavailability of supplemented L-carnitine in patients with IVA who were controlled with glycine and carnitine supplementation and low-protein diet therapy.

Materials and Methods

We examined two patients with IVA. Case 1 was a 4-year-old girl and case 2 was a 5-year-old boy. Both were diagnosed in the neonatal period with decompensated episode of vomiting and lethargy followed hyperammonemia. They were treated with 1.6–1.7 g/kg/day of low protein diet, 40–50 mg/kg/day of oral glycine and 40 mg/kg/day of oral L-carnitine. They had been clinically well on this regimen and admitted for this study. Informed consents for our study were obtained from each parent in advance. Throughout this program, protein restriction and glycine supplementation were continuously maintained as previously. Preceding the study, L-carnitine was discontinued for 3 days; then, following an overnight fast, 50 mg/kg of L-carnitine was given intravenously over 1 hr. Serum and urine samples were collected serially over the next 24 hr. Unfortunately, serum and urine sample of case 2 before intravenous carnitine loading could not be obtained. After 2 or 3 carnitine-free days, oral L-carnitine supplementation was started and increased every 4 days from 25 to 50, 75 and 100 mg/kg/day, as shown in Fig. 2. Morning urine was collected for last two days and serum sample was collected on the last day of each loading dose.

Free and total carnitine concentrations in serum and urine were measured by the method using carnitine dehydrogenase and acylcarnitine hydrolase (Takahashi et al. 1994).

Urinary acylcarnitine profile was determined by carboxylic acid analyzer with an ODS reverse phase column, according to the modified method of Kidouchi et al. (1987). Isovalerylglycine was quantified by carboxylic analyzer and isovalerylglucuronide was determined by liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS). Hippurate was quantified by high performance liquid chromatography (HPLC).

Results

Serum free and total carnitine concentrations prior to this study were below the normal range, and serum acyl/total carnitine ratio was elevated in both patients (Table 1).

TABLE 1. *Baseline levels of serum carnitine before study*

	Total carnitine (nmol/ml)	Free carnitine (nmol/ml)	Acyl/total ratio (%)
Case 1	24.7	12.0	51.4
Case 2	23.1	13.0	45.9
Normal	61.8±13.1*	45.6±11.0*	26.0±10.0*

*Mean±S.D.

Intravenous L-carnitine loading study

Injection of L-carnitine resulted in rapid and remarkable increase in serum free and acylcarnitine levels in both cases, which fell again to a deficient level after 24 hr in case 2, but remained within normal range in case 1 (Table 2). Urinary excretion of free and acylcarnitine also increased remarkably soon after the injection, then decreased promptly; the reduction of acylcarnitine was slower than that of free carnitine (Table 2). Fluctuation of urinary isovalerylcarnitine, which accounted for most of acylcarnitine, was similar to that of acylcarnitine fraction. Acetylcarnitine was excreted only at the peak of acylcarnitine (Fig. 1). Isovalerylglycine was excreted largely throughout the loading, whose excretion pattern was different from that of isovalerylcarnitine in both cases: namely,

TABLE 2. *Carnitine and hippurate during i.v. carnitine loading*

	Serum (nmol/ml)			Urine (mmol/g cre)			
	T.C.	F.C.	A.C.	T.C.	F.C.	A.C.	Hippurate
Case 1							
Before	—	—	—	1.48	0.23	1.25	2.26
1 hr	1290.0	1180.6	109.3	53.47	45.56	7.91	1.75
2 hr	385.9	334.5	51.3	76.43	56.93	19.50	1.01
6 hr	153.4	118.7	34.7	18.49	2.90	15.59	0.45
12 hr	162.5	144.4	18.2	2.79	0.41	2.38	0.21
24 hr	90.3	72.9	17.4	4.11	0.50	3.61	1.08
Case 2							
Before	—	—	—	—	—	—	—
1 hr	1291.5	305.9	985.5	31.37	13.24	17.13	0.51
2 hr	—	—	—	20.52	7.58	12.94	0.51
6 hr	161.8	127.2	34.6	6.88	1.02	5.86	2.24
12 hr	40.4	20.4	19.9	2.83	0.21	2.62	0.14
24 hr	28.3	13.1	15.2	2.09	0.17	1.92	0.72

T.C., total carnitine; F.C., free carnitine; A.C., acylcarnitine; —, Samples not obtained.

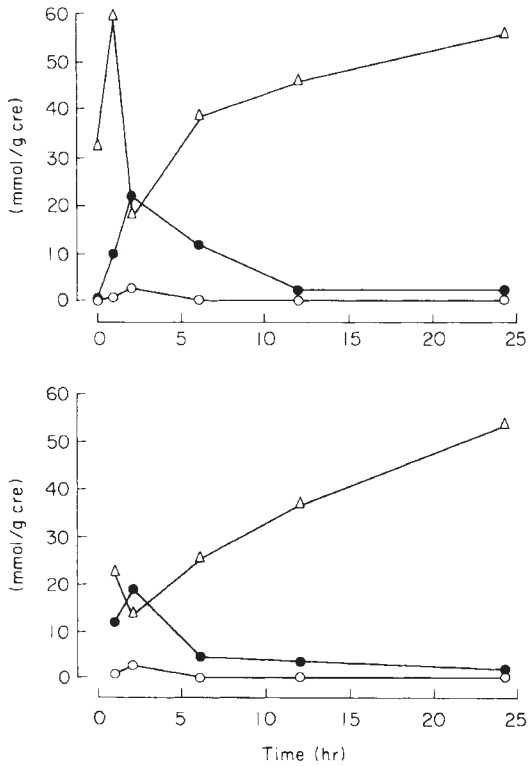

Fig. 1. 50 mg/kg of L-carnitine was loaded intravenously over 1 hr.
Urinary excretion was measured serially. Case 1 (upper) and Case 2 (lower).
○—○, Acetylcarnitine; ●—●, Isovalerylcarnitine; △—△, Isovalerylglycine.

transient decrease in excretion of isovalerylglycine was recognized just at the peak period of isovalerylcarnitine excretion, followed by a gradual increase to 2–3 times the pre-loading level after 24 hr (Fig. 1). No isovalerylglucuronide was detected in the urine. Excretion of hippurate was varied with no relation to carnitine dose.

Oral L-carnitine supplementation study

Results were similar in both cases. Serum free and acylcarnitine concentrations elevated in a dose-dependent manner (Table 3). Urinary excretion of total carnitine increased to 5–10 fold the pre-treatment level by 100 mg/kg of L-carnitine supplement, in which more acylcarnitine was excreted than free carnitine (Table 3), and most of the acylcarnitine was isovalerylcarnitine. Correspondingly, enhanced excretion of isovalerylglycine was observed in spite of the constant glycine dose and protein intake, reaching 2-3 times the initial level by the end of study (Fig. 2). Urinary acetylcarnitine was detectable only with high dose L-carnitine. Glucuronide derivative of isovaleryl-CoA was not detected in the urine. L-Carnitine dose had no influence on urinary hippurate excretion.

Discussion

Secondary carnitine deficiency has been reported in variable metabolic

TABLE 3. *Carnitine and hippurate during oral carnitine supplement*

Carnitine dose (mg/kg)	Serum (nmol/ml)			Urine (mmol/g. cre)			
	T.C.	F.C.	A.C.	T.C.	F.C.	A.C.	Hippurate
Case 1							
0	—	—	—	0.80	0.07	0.73	0.90
	27.56	13.74	13.82	1.02	0.08	0.94	1.36
25	—	—	—	1.15	0.13	1.02	0.87
	40.04	25.08	14.96	1.13	0.15	0.98	0.62
50	—	—	—	1.81	0.26	1.55	2.42
	49.90	33.46	16.44	1.06	0.21	0.85	1.08
75	—	—	—	1.09	0.14	0.95	5.24
	46.08	27.88	18.20	1.37	0.13	1.24	2.75
100	—	—	—	3.16	0.44	2.73	3.65
	58.18	32.60	25.58	3.27	0.45	2.82	0.98
Case 2							
0	—	—	—	0.27	0.02	0.25	0.34
	20.00	11.20	8.80	0.19	0.17	0.02	0.32
25	—	—	—	0.46	0.08	0.38	0.10
	49.58	35.82	13.76	0.61	0.09	0.02	0.37
50	—	—	—	1.34	0.16	1.18	1.00
	44.86	27.74	17.12	1.38	0.18	1.20	0.71
75	—	—	—	1.44	0.16	1.28	0.16
	66.10	41.70	24.40	1.89	0.24	1.65	0.17
100	—	—	—	4.18	0.39	3.79	1.08
	43.90	22.80	21.10	5.16	0.63	4.53	1.22

T.C., total carnitine; F.C., free carnitine; A.C., acylcarnitine; —, Samples not obtained.

defects, such as propionic acidemia, methylmalonic acidemia, mitochondrial β-oxidation defects, glutaric aciduria type 1 and type 2 as well as IVA (Chalmers et al. 1983; Stanley et al. 1983). Among these disorders, L-carnitine supplementation has been advocated as an additional therapy to prevent carnitine deficiency, to regulate imbalanced intramitochondrial acyl-CoA profile, and to eliminate accumulated toxic acyl-CoA as a corresponding acylcarnitine into the urine. However, recent reports have shown some doubts on the efficacy of carnitine therapy (Treem et al. 1989), suggesting the possible inhibitory effect on glycine conjugation in medium chain acyl-CoA dehydrogenase deficiency (Rinaldo et al. 1993), for instance.

In IVA glycine supplementation has been introduced for maintenance treatment to enhance the removal of accumulated isovaleryl-CoA as isovalerylglycine into urine (Krieger and Tanaka 1976; Yudkoff et al. 1978; Elsas and Naglak

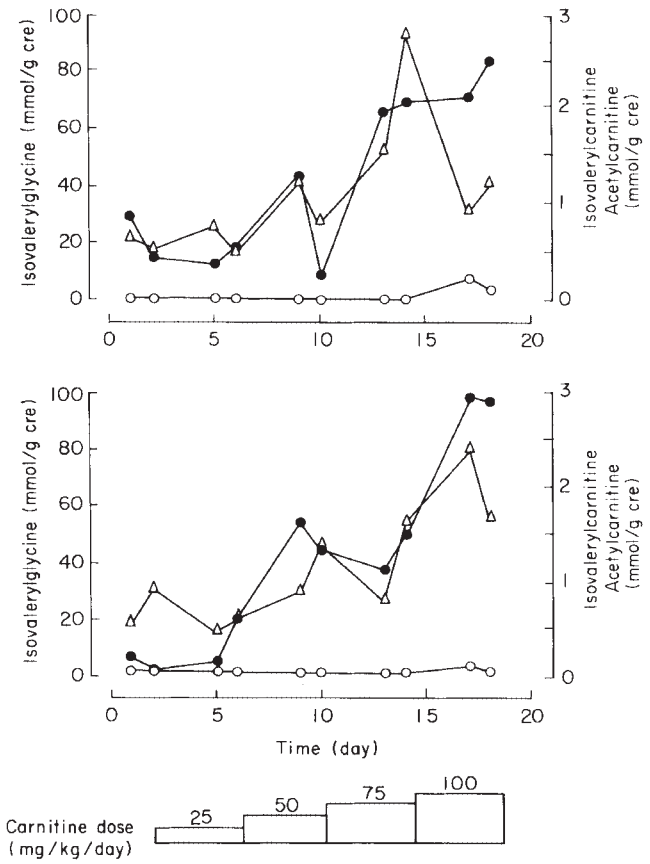

Fig. 2. L-Carnitine was supplemented orally increasing by every 4 days from 25 to 50, 75 and 100 mg/kg/day. Morning urine was collected for last two days and urinary excretion was measured serially. Case 1 (upper) and Case 2 (lower).
○—○, Acetylcarnitine; ●—●, Isovalerylcarnitine; △—△, Isovalerylglycine.

1988; Naglak et al. 1988). But an excessive dose of glycine may bring about hyperglycinemia, and some investigators have recommended supplementation of carnitine instead of glycine (Roe et al. 1984; de Sousa et al. 1986; Mayatepek et al. 1991). However, influence of carnitine on glycine and isovaleryl-CoA metabolism and appropriate therapeutic dose of L-carnitine remain poorly understood.

In intravenous high dose L-carnitine administration study, reverse reaction was demonstrated between isovalerylcarnitine and isovalerylglycine excretion. This suggested that administrated carnitine first combines with isovaleryl-CoA by carnitine acyltransferase, in competition with glycine conjugation by glycine N-acylase, resulting in transient falling isovalerylglycine excretion. After that, along with the lowering of the serum carnitine level, excretion of isovalerylglycine was again enhanced to achieve 2–3 times the pre-loading level by 24 hr. These changes suggest stronger affinity of glycine N-acylase to isovaleryl-CoA than that of carnitine acyltransferase to isovaleryl-CoA. Furthermore, later stimulated excretion of isovalerylglycine could be explained as restoration of intramitochondrial deficient free CoA by carnitine supplementation. This may suggest glycine

dose supplied in this study was not adequate to yield free CoA to presenting normal mitochondrial function independently and additional carnitine supplementation could restore intramitochondrial shortage of free CoA. Actually, the following oral L-carnitine study showed similar findings, though increase of urine isovalerylcarnitine excretion was considerably less than that of intravenous carnitine administration because of partial absorption of orally administrated carnitine. That is, increase in carnitine dose stimulated more isovalerylglycine excretion than isovalerylcarnitine with constant glycine dose and protein intake. The amount of isovalerylglycine excretion was equivalent to that of the high-dose glycine supplementation reported by Elsas and Naglak (1988). Thus, it is likely that more important role of carnitine therapy for a patient with IVA is to provide sufficient free CoA in tissues, promoting isovaleryl-CoA production and isovalerylglycine formation. We attempted to demonstrate increased free CoA by quantifying urinary hippurate excretion. But actually, hippurate excretion did not increase after carnitine loading in our cases. This might be due to minimal benzoate intake in the food and they were well controlled. Meanwhile direct detoxifying effect by isovalerylcarnitine formation might be limited in good conditions without vast isovaleryl-CoA accumulation.

As for an optimal dose of L-carnitine, Berry et al. (1988) reported good results in 4 patients with IVA who were treated with 50 mg/kg of L-carnitine and 180–250 mg/kg of glycine. In this study, additional carnitine supplementation did not stimulate isovalerylglycine excretion. Mayatepek et al. (1991) reported a long-term followed case treated with 60–100 mg/kg L-carnitine and no supplemental glycine. In their case the excretion of short chain acylcarnitine was largely dependent on L-carnitine dose, but isovalerylglycine excretion was slightly decreased during the treatment. Isovalerylcarnitine was not assayed in their report. In a case of de Sousa et al. (1986) oral administration of 200 mg/kg of L-carnitine in addition to 300 mg/kg of glycine resulted not only in increasing isovalerylcarnitine excretion but also doubling of isovalerylglycine excretion. Recently, Van Hove et al. (1994) reported that increasing dose of intravenous L-carnitine did not enhance excretion of isovalerylcarnitine. On the contrary, it caused decrease in excretion of isovalerylglycine. They did not administer glycine. These differences in reaction to carnitine administration may result from the supplemental carnitine and/or glycine dose. Isovalerylglycine is main detoxicating substance of isovaleryl-CoA and glycine supplementation is essential with the dose not causing extreme hyperglycinemia.

In the present investigation, we detected acetylcarnitine in the urine from both of the cases under study just when high-dose of L-carnitine was administered. It suggests that excessive amount of carnitine over the turnover of isovaleryl-CoA might encourage the formation of acetylcarnitine. We observed similar results in propionic acidemia and methylmalonic acidemia (unpublished data). That is, acetylcarnitine was detected in urine only when carnitine was supplemented

sufficiently and reflects sufficient acetyl-CoA formation. Suitable dose of L-carnitine supplementation resulted in increase of acetyl-CoA accompanied by restoring altered energy metabolism during chronic hyperammonemic condition in mice (Ratnakumari et al. 1993). Analysis of urinary acetylcarnitine may be reasonable for determining the optimal dose of L-carnitine administered for each case.

We concluded that 50–100 mg/kg of oral L-carnitine supplementation combined with glycine is necessary for the long-term treatment of patients with IVA, and that it is important to monitor excretion of urinary acetylcarnitine in addition to the routine measurement of serum and urine carnitine and organic acids concentration.

References

1) Berry, G.T., Yudkoff, M. & Segal, S. (1988) Isovaleric acidemia: Medical and neurodevelopmental effects of long-term therapy. *J. Pediatr.*, **113**, 58–64.
2) Chalmers, R.A., Roe, C.R., Tracey, B.M., Stacey, T.E., Hoppel, C.L. & Millington, D.S. (1983) Secondary carnitine insufficiency in disorders of organic acid and metabolism: Modulation of acyl-CoA/CoA ratios by L-carnitine in vivo. *Biochem. Soc. Trans.*, **11**, 724–725.
3) de Sousa, C., Chalmers, R.A., Stacey, T.E., Tracey, B.M., Weaver, C.M. & Bradley, D. (1986) The response to L-carnitine and glycine therapy in isovaleric acidemia. *Eur. J. Pediatr.*, **144**, 451–456.
4) Elsas, L.J. & Naglak, M. (1988) Acute and chronic-intermittent isovaleric acidemia: Diagnosis and glycine therapy. *Acta Paediatr. Jpn.*, **30**, 442–451.
5) Kidouchi, K., Sugiyama, N., Morishita, H., Wada, Y., Nagai, S. & Sakakibara, J. (1987) Analytical method for urinary glutarylcarnitine, acetylcarnitine and propionylcarnitine with a carboxylic acid analyzer and a reversed-phase column. *J. Chromatogr.*, **423**, 297–303.
6) Krieger, I. & Tanaka, K. (1976) Therapeutic effects of glycine in isovaleric acidemia. *Pediatr. Res.*, **10**, 25–29.
7) Mayatepek, E., Kurczynski, T.W. & Hoppel, C.L. (1991) Long-term L-carnitine treatment in isovaleric acidemia. *Pediatr. Neurol.*, **7**, 137–140.
8) Naglak, M., Salvo, R., Madsen, K., Dembure, P. & Elsas, L. (1988) The treatment of isovaleric acidemia with glycine supplement. *Pediatr. Res.*, **24**, 9–13.
9) Ratnakumari, L., Qureshi, I.A. & Butterworth, R.F. (1993) Effect of L-carnitine on cerebral and hepatic energy metabolites in congenitally hyperammonemic sparse-fur mice and its role during benzoate therapy. *Metabolism*, **42**, 1039–1046.
10) Rinaldo, P., Schmidt-Sommerfeld, E., Posca, A.P., Heales, S.J.R., Woolf, D.A. & Leonard, J.V. (1993) Effect of treatment with glycine and L-carnitine in medium-chain acyl-coenzyme A dehydrogenase deficiency. *J. Pediatr.*, **112**, 580–584.
11) Roe, C.R., Millington, D.S., Maltby, D.A., Kahler, S.G. & Bohan, T.P. (1984) L-Carnitine therapy in isovaleric acidemia. *J. Clin. Invest.*, **74**, 2290–2295.
12) Stanley, C.A., Hale, D.E., Whiteman, D.E.H., Coates, P.M., Yudkoff, M., Berry, G.T. & Segel, S. (1983) Systemic carnitine deficiency in isovaleric acidemia. *Pediatr. Res.*, **17**, Suppl. 296 A.
13) Takahashi, M., Ueda, S., Misaki, H., Sugiyama, N., Matsumoto, K., Matsuo, N. & Murao, S. (1994) Carnitine determination by an enzymatic cycling method with carnitine dehydrogenase. *Clin. Chem.*, **40**, 817–821.
14) Tanaka, K. & Isselbacher, K.J. (1967) The isolation and identification of N-

isovalerylglycine from urine of patients with isovaleric acidemia. *J. Biol. Chem.*, **242**, 2966–2972.

15) Tanaka, K., Budd, M.A., Efron, M.L. & Isselbacher, K.J. (1966) Isovaleric acidemia: A new genetic defect of leucine metabolism. *Proc. Natl. Acad. Sci. USA*, **56**, 236–242.

16) Treem, W.R., Stanley, C.A. & Goodman, S.I. (1989) Medium-chain acyl-CoA dehydrogenase deficiency: Metabolic effects and therapeutic efficacy of long-term L-carnitine supplementation. *J. Inherit. Metab. Dis.*, **12**, 112–119.

17) Van Hove, J.L.K., Kahler, S.G., Millington, D.S., Roe, D.S., Chace, D.H., Heales, S.J.R. & Roe, C.R. (1994) Intravenous L-carnitine and acetyl-L-carnitine in medium-chain acyl-Coenzyme A dehydrogenase deficiency and isovaleric acidemia. *Pediatr. Res.*, **35**, 96–101.

18) Yudkoff, M., Cohn, R.M., Puschak, R., Rothman, R. & Segal, S. (1978) Glycine therapy in isovaleric acidemia. *J. Pediatr.*, **92**, 813–817.