Alteration of urinary carnitine profile induced by benzoate administration

Toru Sakuma

Abstract

To study the effect of sodium benzoate on carnitine metabolism, the acylcarnitine profile in the urine of five normal volunteers and two patients with urea cycle disorders was examined with fast atom bombardment-mass spectrometry. The volunteer subjects were given 5 g of sodium benzoate orally and the two patients with urea cycle disorders (carbamyl phosphate synthetase deficiency type I and ornithine transcarbamylase deficiency) were already undergoing treatment with sodium benzoate and L-carnitine. The amount of benzoylcarnitine excretion depended on the dose of both sodium benzoate and L-carnitine in a reciprocal relation. Increased excretions of acetylcarnitine and propionylcarnitine were also noted after sodium benzoate administration. The alteration of the urinary aclycarnitine profile was consistent with the change of mitochondrial CoA profile predicted by in vitro studies of an animal model. It is suggested that urinary acylcarnitine analysis is important to assess the effect of benzoate administration on mitochondrial function in vivo. Supplementation with carnitine may be necessary to minimise the adverse effects of sodium benzoate treatment in hyperammonaemia.

Sodium benzoate is commonly used for the treatment of hyperammonaemia and its toxicity at the usual dose is said to be low.[1 2] However, hypocarnitinaemia[3] and the possibility of benzoate toxicity[4 5] have been recently reported in several cases after sodium benzoate administration. Recently we have detected benzoylcarnitine in the urine of a patient with carbamyl phosphate synthetase deficiency type I who was being treated with sodium benzoate and L-carnitine.[6] The excess excretion of benzoylcarnitine may be the cause of the secondary carnitine deficiency that is often seen in hyperammonaemic patients treated with sodium benzoate.[3]

In this investigation the effect of benzoate administration on carnitine metabolism was assessed in more detail on the basis of the analysis of the urinary acylcarnitine profile. This profile has been considered to reflect the state of mitochondrial acylcarnitine compounds in tissues. The subjects were five volunteers given sodium benzoate and two children with urea cycle disorders taking sodium benzoate and L-carnitine for the management of their hyperammonaemia.

Department of Paediatrics, Nagoya City University Medical School, 1 Miziho-cho, Mizuho-ku, Nagoya 467, Japan

Correspondence to: Dr Sakuma.

Accepted 21 February 1991

Case reports

Case 1 was a 4 year old Japanese boy of unrelated parents. At the age of 3 days he was transported to our hospital because of coma with pulmonary haemorrhage. On admission his blood ammonia concentration was appreciably raised (503 μmol/l) but his urinary orotate was not increased. For the analysis of urea cycle enzyme activities[7] a liver biopsy was performed at the age of 3 months, which confirmed a diagnosis of carbamyl phosphate synthetase deficiency type I (0·1% of control activity). His blood ammonia concentration was maintained at <30 μmol/l by a restricted protein diet, arginine, and sodium benzoate until the time of the study when the patient was aged 4 years.

The dose of intravenous sodium benzoate was gradually reduced from 100 to 50 mg/kg/day as shown in fig 1. There was a rise of blood

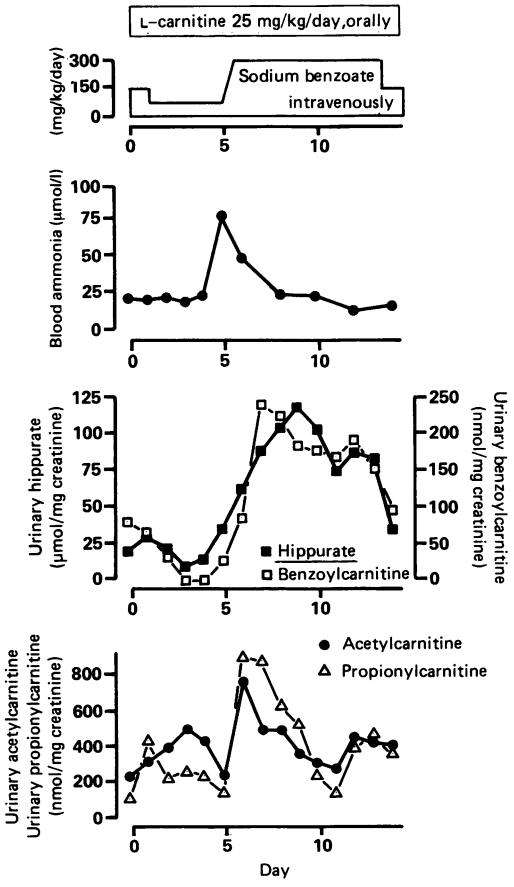

Figure 1 The clinical course of case 1 whose urine samples were examined to assess the appropriate dose of sodium benzoate. The concentration of acetylcarnitine and propionylcarnitine were increased more quickly but transiently compared with that of benzoylcarnitine.

ammonia to 87 μmol/l. The dose of sodium benzoate was then increased to 300 mg/kg/day; this led to a fall in blood ammonia to within the normal range. Throughout the period of study the child remained asymptomatic and was maintained on intravenous L-carnitine at the dose of 25 mg/kg/day.

Case 2 was a 2 year old Japanese boy of unrelated parents. He presented at the age of 2 days with lethargy and convulsions. His blood ammonia concentration was 334 μmol/l and urinary orotate was remarkably increased (91·5–354·0 μg/mg creatinine). The concentration of plasma citrulline was decreased. The diagnosis of ornithine transcarbamylase deficiency was established on the basis of enzyme assay of a liver biopsy specimen (activity 1% of control). He was treated with a low protein diet and sodium benzoate (200 mg/kg/day). He remained asymptomatic and has shown normal growth. When he was 2 years old, hypocarnitinaemia of 1·0–3·0 μmol/l was noticed and an L-carnitine supplement of 50 mg/kg/day was started.

Five male volunteers who were on no medication and in good health were enrolled in the study to assess the short term effect of sodium benzoate administration. Informed consent was obtained from all of them. Their mean (SD) age was 23 (2) years and they weighed 65 (5) kg. Sodium benzoate (5 g) was orally administered after overnight fasting. Blood and urine samples were taken at various intervals (0, 2, 4, 6, and 24 hours after the load).

Methods

All serum and urine samples were stored at −20°C for subsequent analysis. Blood ammonia concentration was measured by the glutamate dehydrogenase method. Urinary hippurate was assayed by the colorimetric method.[8] All the reagents used were of reagent grade.

URINARY ACYLCARNITINE PROFILE

D3-carnitine ($^2H_3$-Me_1-N:carnitine) was the kind gift of the Earth Chemical Company, Hyogo, Japan. Acetyl-D3-carnitine and propionyl-D3-carnitine were synthesised with each acylchloride and D3-carnitine by a similar method to the benzoyl-D3-carnitine synthesis described previously.[6] These authentic acyl-D3-carnitines were over 95% in purity when assayed by the carboxylic acid analyser with an ODS column.[9] Urinary concentrations of carnitine derivatives, whose methyl esterification were done by deuterium exchanged methanol (CD30D), were quantified with fast atom bombardment-mass spectrometry (JMS-DX 300; JMA-DA 3500, JEOL, Tokyo, Japan) by the stable isotope dilution techniques as described by Montgomery and Mamer.[10] By this analytical procedure, because the one fragment ion signal of both labelled and unlabelled benzoylcarnitine-D3-methyl ester (M-62, M-59, respectively) is just the same as that of acetyl-D3-carnitine-D3-methyl ester (m/z=224), these two compounds are indistinguishable. Additionally, the maximal strength of each acylcarnitine signal has a different scanning time. To make a quantitative analysis of acylcarnitine, each acyl-D3-carnitine should be used as its own internal standard. For a quantification of acetyl, propionyl, and benzoylcarnitine, therefore, acetyl-D3-, propionyl-D3-, or benzoyl-D3-carnitine was used as an internal standard in each sample. By this procedure the calibration curve of acetyl, propionyl, and benzoylcarnitine was linear over the sample concentration range from 1 to 500 nmol/ml.

STATISTICAL ANALYSIS

Each value represented the mean (SE). Values of the two groups were compared with the Student's t test. Unless indicated otherwise the level of significance was set at $p<0·05$.

Results

In case 1 a slightly reduced urinary excretion of hippurate was noted after lowering the dose of sodium benzoate (50 mg/kg/day). This rapidly increased again when the dose was augmented to 300 mg/kg/day. The excretion pattern of benzoylcarnitine was almost the same as that of hippurate (fig 1). Acetylcarnitine and propionylcarnitine concentrations in urine also changed similarly to benzoylcarnitine, but their amounts were reduced gradually even though the same dose of sodium benzoate was given.

In case 2, as shown in fig 2, a large amount of propionylcarnitine and acetylcarnitine but only a small amount of benzoylcarnitine (43 μmol/g creatinine) was excreted in urine when sodium benzoate was given without L-carnitine administration. Benzoylcarnitine excretion increased to 10-fold by the addition of L-carnitine and the hippurate excretion also increased to a lesser degree.

In the volunteers the excretion of acetylcarnitine and propionylcarnitine increased rapidly and reached the maximum point at two hours after loading (that is, 7·5-fold and 22-fold, respectively). The excretion of both hippurate and benzoylcarnitine were slightly slow and reached the maximum at four hours after loading (table).

Carnitine derivatives and hippurate in urine after sodium benzoate loading test in volunteers. Results are mean (SE)

	Time after load (hours)				
	0	*2*	*4*	*6*	*24*
Acetylcarnitine (nmol/mg creatinine)	13·1 (4·2)	97·8 (23·2)*	94·7 (26·4)*	28·4 (10·2)	28·5 (4·9)
Propionylcarnitine (nmol/mg creatinine)	9·0 (2·4)	200·0 (41·5)*	179·3 (52·4)*	20·9 (3·7)	11·8 (4·3)
Benzoylcarnitine (nmol/mg creatinine)	1·9 (0·4)	34·2 (13·2)*	67·4 (21·5)*	51·8 (18·2)	3·1 (0·7)
Hippurate (μmol/mg creatinine)	3·9 (0·6)	205·0 (41·7)*	221·1 (47·8)*	32·2 (7·2)*	2·2 (0·4)

*Significant difference from baseline.

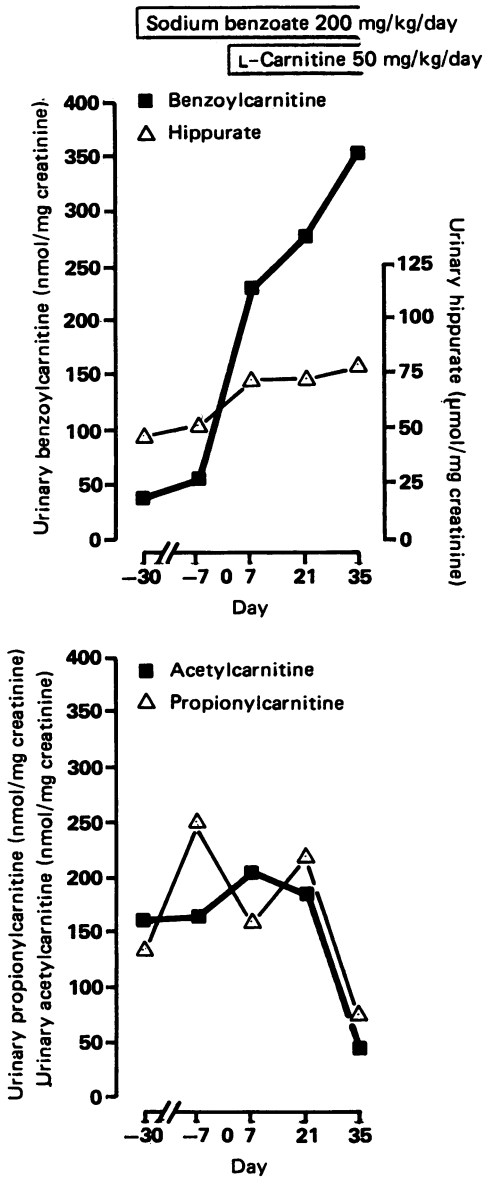

Figure 2 Case 2: treated with oral sodium benzoate at the dose of 200 mg/kg/day, L-carnitine was started from day 0 at 50 mg/kg/day. Upper graph: benzoylcarnitine excretion was increased about 10 times by the carnitine supplementation and more hippurate was also excreted than at 0 hours. Lower graph: both concentrations were very high but were replenished after L-carnitine supplement.

Discussion

Because benzoyl-CoA conjugates with glycine to be excreted as hippurate into the urine, sodium benzoate has been used for the treatment of hyperammonaemia.[1 2] Recently we reported that a certain amount of benzoylcarnitine was excreted in the urine of a patient with carbamyl phosphate synthetase deficiency type I on benzoate treatment.

In this investigation a relatively large amount of benzoylcarnitine could be detected in urine of volunteers receiving sodium benzoate as well as from two patients with urea cycle disorders. Therefore, benzoylcarnitine might be another important product (0·5 to 7%) of benzoate and its excretion in urine is a possible cause of hypocarnitinaemia associated with long term benzoate treatment for hyperammonaemic patients. In addition, increased acetylcarnitine and propionylcarnitine excretions were also recognised both in patients and volunteers. The sum of these three carnitine derivatives accounted for the majority of increased esterified carnitines. Thus the increase in excretion of acetylcarnitine and propionylcarnitine, plus benzoylcarnitine excretion, might be another cause of hypocarnitinaemia in benzoate treatment.

These phenomena could be explained as follows. Acyl-CoAs were catalysed with carnitine then formed corresponding acylcarnitines according to the equation below:

$$[\text{acyl-CoA}][\text{carnitine}] = k\,[\text{acylcarnitine}][\text{CoA-SH}]$$

where k is a constant. As amounts of acylcarnitine were excreted into the urine after benzoate administration in volunteers and in patients, both the increased influx of carnitine into mitochondria and the decrease of the acyl-CoA concentration were predicted, which is consistent with the data of in vitro study on rats.[11 12]

An unexpected observation was the finding of large amounts of propionylcarnitine in urine rather than acetylcarnitine. Although this important change was induced by the administration of sodium benzoate, the cause of it was obscure.

Sodium benzoate has been administered to hyperammonaemic patients. It is also important not to overlook the adverse potential for mitochondrial functions and to administer it appropriately. In the present study it was confirmed that the alteration of carnitine metabolism could generally be induced by benzoate administration and that its analysis might be useful to examine the effect of benzoate on mitochondria in vivo. These results suggested that L-carnitine supplementation should be considered to correct a carnitine shortage as well as to preserve an adequate intramitochondrial CoA profile.

Thanks go to Dr Naruji Sugiyama, Dr Takashi Ichiki, Dr Masanori Kobayashi, and Dr Yoshiro Wada for discussion of the manuscript. Thanks are also due to Dr Yoshimi Suzuki and Dr Yutaka Nishimura (department of paediatrics, Toyohashi City Hospital) for providing specimens from case 2.

1 Brusilow SW, Valle DL, Batshaw ML. New pathway of nitrogen excretion in inborn errors of urea synthesis. *Lancet* 1979;ii:452–4.
2 Brusilow SW, Tinker J, Batshaw ML. Amino acid acylation: a mechanism of nitrogen excretion in inborn errors of urea synthesis. *Science* 1980;207:659–61.
3 Mazuda I, Ohtani Y, Ohyanagi K, *et al.* Hyperammonemia related to carnitine metabolism with particular emphasis on ornithine transcarbamylase. *Enzyme* 1987;38:251–5.
4 O'Connor JE, Costell M, Glisolia S. The potentiation of ammonia toxicity by sodium benzoate is prevented by L-carnitine. *Biochem Biophys Res Commun* 1987;145:817–24.
5 Cyr DM, Maswoswe SM, Tremblay GC. Inhibition of the urea cycle and de novo pyrimidine biosynthesis by sodium benzoate. *J Inherited Metab Dis* 1987;10:308–10.
6 Sakuma T, Asai H, Ichiki T, *et al.* Identification of benzoylcarnitine in the urine of a patient of hyperammonaemia. *Tohoku J Exp Med* 1989;159:147–51.
7 Cohen NS, Cheung CW, Raijman L. The effects of ornithine on mitochondrial carbamyl phosphate synthesis. *J Biol Chem* 1980;255:10248–55.
8 Tomokuni K, Ogata M. Direct colorimetric determination of hippuric acid in urine. *Clin Chem* 1972;18:349–52.
9 Kidouchi K, Sugiyama N, Morishita H, *et al.* Identification of glutarylcarnitine in glutaric aciduria type 1 by carboxylic acid analyzer with an ODS reverse-phase column. *Clin Chim Acta* 1987;164:261–6.
10 Montgomery JA, and Mamer OA. Measurement of urinary free and acylcarnitine: quantitative acylcarnitine profiling in normal humans and in several patients with metabolic errors. *Anal Biochem* 1989;176:85–95.
11 McCune SA, Drunt PJ, Flanders LE, *et al.* Inhibition of hepatic gluconeogenesis and lipogenesis by benzoic acid, p-tetr-butylbenzoic acid, and a structurally related hypolipidemic agent SC-33459. *Arch Biochem Biophys* 1982;214:124–33.
12 Swartzentruber MS, Harris RH. Inhibition of metabolic processes by coenzyme-A-sequestering aromatic acids. *Biochem Pharmacol* 1987;36:3147–53.