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In vivo propionate oxidation as a prognostic indicator in disorders of propionate metabolism

G.N. Thompson[1], J.H. Walter[2], J.-L. Bresson[3] J.-P. Bonnefont[3] J.-M. Saudubray[3], J.V. Leonard[2], and D. Halliday[1]

[1]Nutrition Research Group, Clinical Research Centre, Harrow, UK
[2]Department of Child Health, Institute of Child Health, London, UK
[3]Clinique Genetique Medicale, Hôpital des Enfants Malades, Paris, France

Abstract. Biochemical markers such as plasma and urinary metabolite concentrations and in vitro enzyme activity are of limited prognostic value in the most common disorders of propionate metabolism, methylmalonic acidaemia (MMA) and propionic acidaemia (PA). In vivo propionate oxidation was compared with conventional prognostic measures as predictors of clinical severity in seven children with MMA and six with PA. Propionate oxidation was measured using a continuous infusion of [1-^{13}C]propionate and was expressed as the rate of appearance of $^{13}CO_2$ as a percentage of the propionate infusion rate. Children with MMA (mean oxidation 51.2%, range 17.5–91.6, $P < 0.05$) and with PA (mean oxidation 36.3%, range 3.0–91.1, $P = $ NS) oxidised substantially less propionate than controls (mean oxidation 81.9%, range 69.4–101.0, $n = 5$). Percentage oxidation was a better predictor of the clinical severity score ($r = 0.75$, $P < 0.01$) than was in vitro enzyme activity, plasma propionate or methylmalonate concentration or urinary metabolite excretion. Studies were repeated after an interval of 1–3 weeks in six of the subjects; the percentage oxidation in each subject was virtually unchanged between studies (coefficient of variation 8.6%). These results suggest that in vivo oxidation measurements using [^{13}C]propionate are both reproducible and prognostically useful in disorders of propionate metabolism.

Key words: Propionate – Stable isotope – Oxidation – Methylmalonic acidaemia – Propionic acidaemia

Introduction

Disorders of propionate metabolism have a wide range of clinical expression varying from severe neonatal presentation with the risk of major handicap and death to mild disease which usually responds well to treatment with good long-term outcome [10]. Prognostic classification of patients at diagnosis is often difficult and is hampered by the poor correlation between clinical severity and in vitro enzyme activity of methylmalonyl-CoA mutase in methylmalonic acidaemia (MMA) and propionyl-CoA carboxylase in propionic acidaemia (PA) [4, 8, 10]. We have measured propionate oxidation in vivo using stable isotope continuous infusion techniques in MMA and PA and established the relationship of this measurement to outcome.

Offprint requests to: G.N. Thompson, The Murdoch Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia

Abbreviations: MMA = methylmalonic acidaemia; PA = propionic acidaemia

Methods

Subjects

Seven children with MMA and six with PA were studied. Diagnosis was established on the basis of clinical features, increased urinary excretion of propionate metabolites and, in some cases, in vitro enzyme assays. Age at presentation and at time of study, sex, dietary valine intake and clinical severity are shown in Table 1. Clinical severity was measured on a scale of 0 (mild) to 9 (severe) as described previously [14]. This scale was based on scores for IQ, growth, feeding pattern, protein tolerance and episodes of acute decompensation. All MMA subjects were unresponsive to vitamin B12 with the exception of subject 7, who was receiving adenosylcobalamin 2 mg IM fortnightly at the time of study. There was no evidence of biotin responsiveness in any subject with PA. Enzyme assays were performed in cultured skin fibroblasts for methylmalonyl-CoA mutase activity in six subjects with MMA and for propionyl-CoA carboxylase activity (in the absence and presence of biotin) in four subjects with PA using standard techniques [2, 3, 6]. Five normal adults (mean age 33 years, range 23–42, 4 male, 1 female) were studied as controls. The studies were approved by the Harrow District Ethical Committee and the Hospitals for Sick Children and Institute of Child Health Standing Committee on Ethical Practice and were performed with the informed consent of the subjects' parents.

Isotopes

Sodium [1-^{13}C]propionate (99% 1-^{13}C) and sodium [^{13}C]bicarbonate (99% ^{13}C) were obtained from Cambridge Isotopes Laboratories (Woburn, Massachusetts, USA). The isotopic purity of sodium [1-^{13}C]propionate was verified by gas chromatography/mass spectrometry.

Table 1. Characteristics of subjects, plasma methylmalonate and propionate concentrations, urinary metabolite excretion and in vitro and in vivo enzyme activities

Subject	Sex	Age at presentation	Age at time of study (years)	Valine intake (mg/kg/24h)	Clinical severity[a]
Propionic acidaemia					
1	M	2 days	5.0	32	8
2	F	3 weeks	2.0	89	1
3	F	1 week	2.4	17	8
4	F	1 week	3.0	66	9
5	M	6 months	0.5	40	2
6	F	20 months	22.1	64	0
Methylmalonic acidaemia					
7	M	3 days	6.6	62	0
8	M	6 days	0.9	55	7
9	M	5 months	6.5	43	7
10	M	4 days	1.4	38	5
11	F	4 months	10.0	58	4
12	M	3 months	5.5	67	1
13	F	2 years	3.6	46	1

Subject	Plasma concentration (μmol/l)		Urinary metabolites	In vitro enzyme activity[c]	In vivo oxidation[e]
	Propionate	Methylmalonate			
1	217			<0.005	23.7
2	7			0.01	91.1
3	120			–	19.3
4	1266			0.01	3.0
5	23			–	38.0
6	20			0.02	42.8
Mean					36.3
7	10	<0.1	17	33[d]	59.1
8	188	0.6	65	6	40.9
9	29	1.8	8	<1	17.5
10	57	<0.1	41	3.1	59.7
11	21	0.9	25	–	28.0
12	24	0.7	46	<1	61.0
13	11	<0.1	8	10.3	91.6
Mean					51.2

[a] Graded on a scale from 0 (mild) to 9 (severe) as described previously [14]

[b] Excretion of methylmalonate, methylcitrates, propionylglycine and 3-hydroxypropionate, μmol/kg per hour

[c] Propionyl-CoA carboxylase activity (PA, nmol/min/per milligram protein, NR 0.33–0.58) and methylmalonyl CoA mutase activity (MMA, % incorporation into protein of [^{14}C]propionate compared with [^{3}H]glycine, NR 21–43) were assayed in cultured skin fibroblasts; in the presence of adenosylcobalamin

[d] Rate of conversion of [^{13}C]propionate to $^{13}CO_2$ expressed as percentage of [^{13}C]propionate infusion rate

Protocol

All subjects were studied when clinically well with the exception of one subject (subject 12) who underwent studies both when well and during an episode of acute metabolic decompensation. Isotope infusions were performed following an overnight fast, as feeding may induce changes in background enrichment of expired CO_2 [12]. The following morning an intravenous cannula was inserted into a peripheral vein for collection of a single blood specimen and subsequently for isotope infusion. After collection of baseline expired air samples, priming bolus doses of [1-^{13}C]propionate (children 0.5 mg/kg, adult controls 0.1 mg/kg] and $Na^{13}CO_3$ (children 0.08–0.2 mg/kg, adults 0.2 mg/kg) were given intravenously. [1-^{13}C]propionate (children 0.5 mg/kg per hour, adults 0.2 mg/kg per hour) was then infused at a constant rate over 4 h. Higher propionate production rates in MMA and PA compared with normal adults [15, 16] necessitated the higher infusion rates in the children. Expired air samples were collected at 15–20 min intervals in the final 2.5 h of each infusion. Total expired air CO_2 production was measured by indirect calorimetry [5] throughout the infusion in six of the subjects and for a period of at least 20 mins in the final 2 h of the infusion in the remaining subjects and controls. In MMA subjects a timed urine collection for measurement of urinary metabolite excretion was made over at least 5 h commencing with the infusion.

Tests were repeated in six subjects (three MMA, three PA, subjects 2, 3, 6, 10, 12 and 13) 1–3 weeks after the initial study to determine the reproducibility of oxidation measurements. One subject (subject 12) underwent a third propionate oxidation study during an episode of acute metabolic decompensation. This episode had been preceded by an upper respiratory infection and at the time of study the child had been treated with intravenous glucose (0.2 g/kg per hour) for 8 h, was irritable but alert, had no evidence of metabolic acidosis and had a plasma methylmalonate concentration of 1.4 mmol/l.

The percentage of [^{13}C]propionate converted to $^{13}CO_2$ was determined from the total CO_2 production rate and the enrichment of $^{13}CO_2$ in expired air. It was assumed that 81% of $^{13}CO_2$ generated in the whole body appeared in expired air [1]. Enrichment of $^{13}CO_2$ in expired air was measured using a Finnigan Delta-E isotope ratio mass spectrometer (Finnigan MAT, Hemel Hempstead, UK). Baseline measurements were performed in duplicate, or in quadruplicate if the initial measurements differed by more than 5%.

Plasma propionate concentration was measured by isotope dilution gas chromatography/mass spectrometry using [$^{2}H_5$]propionate as internal standard in a similar manner to that described previously [16]. In MMA subjects plasma methylmalonate concentration was measured by high performance liquid chromatography [11] and urinary metabolites (methylmalonate, methylcitrate 3R and 3S isomers, propionylglycine and 3-hydroxypropionate) by gas chromatography [4]. Statistical comparisons were made using the Mann-Whitney U test.

Results

Typical plateau measurements of $^{13}CO_2$ enrichment in expired air are shown in Fig. 1. In each of the studies, steady state of $^{13}CO_2$ enrichment was achieved as defined by visual inspection of the plateau and by plateau coefficient of variation of less than 5%. Calculations of propionate oxidation were made from the mean of all sampling points at enrichment plateau after baseline subtraction.

Rates of propionate oxidation in individual subjects are shown in Table 1 and Fig. 2. The mean percentage of propionate oxidised in MMA subjects was 51.2% (range 17.5–91.6) and in PA subjects 36.3% (3.0–91.1). Oxidation rates tended

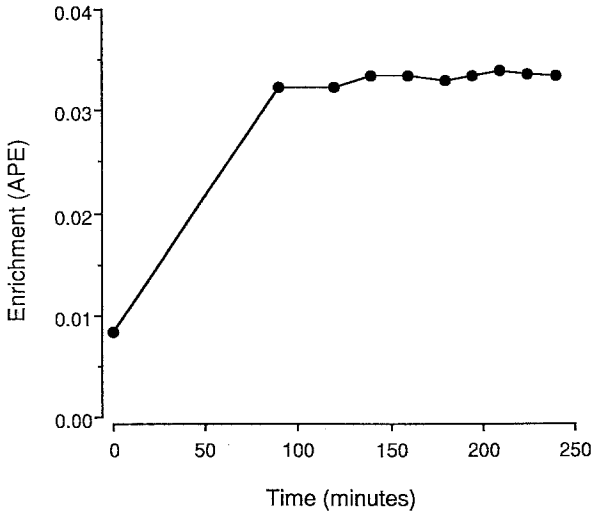

Fig. 1. Typical pattern of expired air $^{13}CO_2$ enrichment measurements during continuous infusion of [1-^{13}C]propionate in the fasted state in a MMA subject

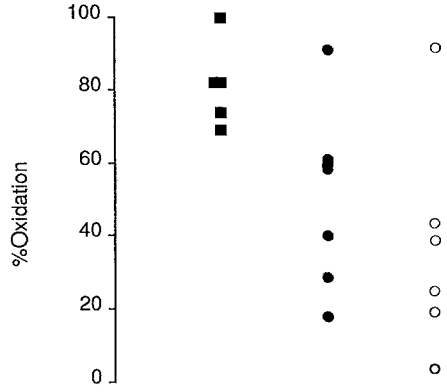

Fig. 2. Percentage oxidation of [^{13}C]propionate in control (■), MMA (●) and PA (○) subjects

Table 2. Reproducibility of in vivo propionate oxidation measurements[a]

Subject	First measurement	Second measurement
2	91.1	92.1
3	19.3	19.5
6	42.8	53.1
10	59.7	65.7
12	61.0	74.3
13	91.6	95.2

[a] Rate of conversion of [^{13}C]propionate to $^{13}CO_2$ expressed as percentage of [^{13}C]propionate infusion rate. Repeat measurements were made after an interval of 1–3 weeks. Coefficient of variation between measurements was 8.6%

to be lower in PA subjects than in those with MMA. Oxidation in both groups was substantially less than in controls (81.9%, range 69.4–101.0, $P < 0.05$ and NS as compared with MMA and PA subjects respectively).

Within individuals, oxidation rates were highly reproducible (coefficient of variation 8.6%, Table 2). Acute metabolic

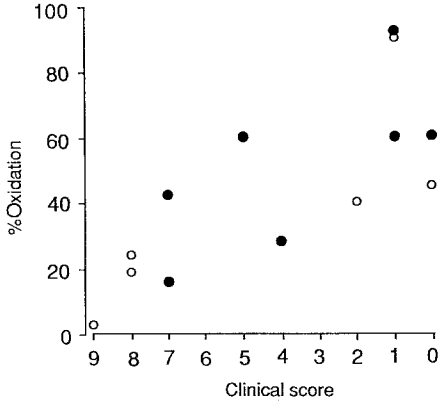

Fig. 3. Relationship between clinical severity score [4] and [^{13}C]propionate oxidation in MMA (●) and PA (○) subjects. $r = 0.75$, $t = 3.78$, $P < 0.01$

Table 3. Regression coefficients between clinical score[a] and in vivo oxidation, substrate concentration, in vitro enzyme activity and urinary metabolite excretion

	n	Regression coefficient	*t*
In vivo oxidation	13	−0.75	3.78
Plasma propionate concentration	13	0.57	2.29
Plasma methylmalonate concentration			
MMA subjects	6	0.61	1.71
In vitro enzyme assay			
MMA subjects	6	−0.57	1.41
MMA and PA subjects	10	−0.42	1.31
Urinary methylmalonate excretion			
MMA subjects	6	0.33	0.79

[a] As described previously [14]

decompensation appeared to have little effect on propionate oxidation; subject 12 oxidised 67% of propionate infused when unwell compared with 61% and 74% when well.

The relationship between clinical severity and percentage oxidation in all subjects is shown in Fig. 3. The oxidation value appeared a better prognostic indicator than in vitro enzyme activity, plasma propionate or methylmalonate concentration or urinary metabolite excretion (Table 3).

Discussion

All subjects with disordered propionate metabolism in the current study were able to oxidise a significant proportion of [1-^{13}C]propionate infused intravenously to expired air $^{13}CO_2$. The incomplete nature of the metabolic defect in vivo contrasts with findings in vitro where, particularly in PA, the enzyme deficiency is often virtually complete [4]. The contrast between in vivo and in vitro metabolism of propionate is vividly demonstrated by comparisons between activities in subjects in whom in vitro assays were performed. Subjects 2, 6 and 12, for example, had relatively mild disease and low clinical scores but markedly decreased propionate carboxylase activity in vitro. In vivo oxidation, on the other hand, reflected more closely their clinical course. The close correlation between clinical score and in vivo propionate oxidation (Fig. 3, Table 3), suggests that this technique may be of prognostic

value in children with inborn errors of propionate metabolism.

The safety of stable isotopes in human studies is well established [7]. The total propionate dose administered in a propionate oxidation study is about 25 μmol/kg, or about 2% of the total daily turnover of propionate in a typical MMA or PA subject [15]. No subject in the current study experienced any side effect as a result of the propionate infusion. Further, all tolerated the procedure without difficulty. Although the youngest patients studied here were 6 months old, the test should be easily performed in children of all ages. The most difficult technical procedure in our experience is the measurement of total CO_2 production rate, which may be limiting particularly between the ages of 1 and 2 years.

The variation in oxidation rate measurements in individual subjects (Table 2) can be accounted for by analysis of errors of the various measurements. Error in measurement of CO_2 production is between 5% and 10%, in infusion rate less than 2% [9], and in estimation of $^{13}CO_2$ enrichment plateau less than 5%. A small error may also exist in the estimate of CO_2 which is excreted in expired air [1]. Once these errors are taken into account, the in vivo propionate oxidation in a given subject would appear to remain relatively constant. Further, the major physiological disturbances which accompany acute metabolic decompensation did not appear to significantly alter the oxidation rate in subject 12. This finding would suggest that propionate accumulation in acute episodes in MMA and PA results from increased production of propionate rather than impaired disposal.

Some subjects with mild clinical manifestations achieved similar percentage oxidation rates to those found in controls (Fig. 2). These subjects would appear to have sufficient oxidative capacity to handle the propionate produced under normal physiological conditions. However, during episodes of metabolic stress such as infection, propionate production may increase so that it exceeds the oxidative capacity. It is likely that the performance of these studies in the unstressed state decreased the discriminatory power against control subjects. For this reason the test would be of limited diagnostic value.

Enzyme assays in cultured skin fibroblasts correlate poorly with clinical course in a number of metabolic conditions [13]. The most likely explanations for these discrepancies are that residual enzyme activity may be present in other tissues and that significant metabolism of the substrate involved in the enzyme block may be achieved through alternate pathways. The exact mechanism involved probably varies with different conditions. In disorders of propionate metabolism the increased urinary excretion of methylcitrate, hydroxypropionate and other metabolites [10] is evidence of increased alternate pathway activity. Alternate pathways would be expected to have greater activity in PA as compared with MMA because of the higher plasma propionate concentrations and greater excretion of propionate metabolites other than methylmalonate. In vivo propionate oxidation, however, tended to be greater in MMA subjects in the current study, suggesting that residual enzyme activity may be responsible for significant propionate metabolism at least in patients with MMA.

In summary, the measurement of propionate oxidation in vivo in MMA and PA may contribute to prognostic classification, as well as providing valuable information about the dynamics of propionate metabolism in these disorders.

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