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Diet treatment of branched chain ketoaciduria studied by proton magnetic resonance spectroscopy

Short Communication

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Summary. A novel nuclear magnetic resonance method is proposed for the diagnosis and follow-up of patients affected by branched chain ketoaciduria. The method allows quantitation of the branched chain amino acids (BCAA's) such as leucine, isoleucine and valine and of related keto- and hydroxy acids by means of a single spectrum. The method implies short time of analysis, as opposed to the very long time required by the techniques currently in use (amino acid analyzer combined with gaschromatography/mass spectrometry of keto- and hydroxyacids), it is easy and suitable for adjustements of the dietary treatment even on a daily basis. The case of a 15 days old newborn child, presenting muscular hypertonicity was unambiguously diagnosed in few minutes by means of one single NMR spectrum of urine. More interestingly, NMR spectra of serum in the following days were suitable for quantitating amino-, and keto acids as well as other metabolites of relevance in the follow up of the dietary treatment of the disease. After a diet lacking of BCAA's, to eliminate keto acids, a low BCAA diet was introduced, that succeeded in keeping the serum levels of the three amino acids within the normal range, while dropping the related keto acids.

Keywords: Amino acids – Maple syrup urine disease – Nuclear magnetic resonance spectroscopy – Diet management – Branched chain amino acids – Branched chain keto acids

Introduction

Branched chain ketoaciduria, a disease also known as "maple syrup urine disease" (MSUD) is characterized by a functional impairement of the branched chain α-keto acid dehydrogenase multienzymatic complex (BCKADH, EC 1.2.4.1), that catalyzes the oxidative decarboxylation of the

branched chain keto acids (BCKA's), yielding the related acylCoA's, at the second step of the catabolism of the three branched chain amino acids (BCAA's), namely leucine, isoleucine and valine (Danner and Elsas, 1989). The impairement of BCKADH interrupts BCAA catabolism after the first transaminating step, leading to increasing serum, urine, CSF and tissue levels of the BCKA's 2-ketoisocaproic (KIC), 2-keto,3-methylvaleric (KMV) and 2-ketoisovaleric (KIV) acids. These keto acids are believed to be neurotoxic and hence responsible of the severe brain damage of MSUD in the absence of early diagnosis and dietary treatment. The diet consists in administrating, during the first years of life, controlled amounts of BCAA's, which are essential amino acids, in order to maintain a normal serum content of the three amino acids, while preventing the related neurotoxic keto acids from accumulating.

A growing body of research concerning proton magnetic resonance spectroscopy applications in clinical chemistry has developed over the past ten years because of its potential for detection of all proton containing metabolites in (and often below) the 100μM range in biological fluids (Pontoni et al., 1994). Among several other inherited diseases, MSUD has already been identified via NMR by means of its typical fingerprint-pattern of the proton NMR spectra of both urine and serum (Iles and Chalmers, 1988; Lehnert and Hunkler, 1986), for the purpose of diagnosis.

Aim of the present paper is not only that of testing NMR as a very rapid and unambiguous diagnostic tool, but, owing to its completeness and rapidity, it is also that of proposing NMR as a viable system to monitor the effects of the diet on BCAA and BCKA levels in biological fluids.

Materials and methods

Urine specimens were collected for 24 hours and 300 µL amounts were brought to pH 2.5 ± 0.03 by means of a Radiometer automatic titration unit equipped with a 2.5 mL automatic burette filled with 3M HCl. Acid environment is required in order to keep pH far from pKa of most organic acids, in order to minimize possible pH dependent slight changes in chemical shift. The sample is then mixed with 100 µL of deuterated water in which a known amount of perdeuterated sodium trimethylsilylpropionate (TSP) was added; the obtained solution was then inserted into a 5 mm NMR tube and analyzed in a Bruker 200 AC-E 4.7 Tesla nuclear magnetic resonance Spectrometer with 200 MHz resonance of proton. TSP is taken as a qualitative standard for chemical shift scale as well as a quantitative external standard for peak area calculations according to Tofts and Wray (1988). The peaks indicated in Fig. 1 were assigned (Lehnert and Hunkler, 1986) and used for quantitation of the metabolites. Area estimates are performed by the computer of the spectrometer. Peak areas of KIC, KMV, Ile and Leu around 1 ppm could not be satisfactorily separated, but could be exploited to obtain estimates of the sum of the two amino acids, being the two keto acids estimatable elsewhere in the spectrum. The signal around 1 ppm can be qualitatively resolved only using a spin-echo sequence, but quantitation under such conditions is reported as very poor (Wevers et al., 1994). For routine analyses, 128 and 512 scans of 3.15 sec are acquired for urine and deproteinized plasma samples, respectively, during the follow-up, although for the mere diagnostic purpose, 32 scans of an urine or serum sample gives already an unambiguous MSUD pattern. The water peak was eliminated by off resonance gate decoupling. All other NMR experimental details are according to Lehnert and Hunkler (1986) with minor modifications.

Results and discussion

Case report and diagnosis

Spectra in Fig. 1 represent the NMR analyses of serum (a), urine (b) and cerebrospinal fluid (c) of a newborn of 15 days of age, weight about 3 Kg, with severe neurologic symptoms, such as hypertonicity, poor feeding with bottle refusal, sleepiness and sweet smelling urine. The serum spectrum of Fig. 1a is by itself unambiguously diagnostic for MSUD, in that peaks assigned to branched chains are clearly detected in abnormous amounts around 1 ppm (Iles and Chalmers, 1988). The peaks assigned to the BCKA's are not detectable in normal subjects (Hoffer et al., 1993), while the normal aminoacidic peaks are even ten times lower. The abnormous levels of BCAA's have been confirmed on two specimens by means of a standard amino acid analysis by HPLC, that gave less than 5% difference with respect to NMR estimates. Even more evident is the presence of the six analytes in the urine (Fig. 1b), while the presence of the three BCKA's in the cerebrospinal fluid (Fig. 1c) is consistent with the proposed neurotoxic effect of such metabolites.

Based on both serum and urine spectra, an unambiguous diagnosis [as opposed to the Guthrie test and to amino acid determination alone, particularly in the first days of life (Snyderman and Sansaricq, 1985)] was made in less than half an hour after the arrival of specimens, a very short time when compared to the established amino acid analyzer and gas/mass analysis or to other cumbersome techniques such as BCKADH assay on cell tissues or whole body ^{13}C-leucine oxidation (Elsas et al., 1993) that are anyhow important in confirming the diagnosis. The 5 minutes time required for a diagnosis based only on urine NMR analysis (that is unambiguous as well) is shorter than that required by a commercially available HPLC test (Pickering Laboratories, Inc.), and gives several further useful information in ascertaining the diagnosis, like the BCKA content, which is more important in diagnosis, being these keto acids normally undetected (Hoffer et al., 1993).

Therapy

As soon as the diagnosis was ascertained, the patient was immediately blood exchanged in the 15th day of life and administered a diet as illustrated in Table 1, without further exchange transfusions; however, one regular transfusion per week was carried out.

Many metabolites have been reported as altered in MSUD (Danner and Elsas, 1989) and can be monitored in patients by NMR, such as BCAA's, BCKA's, branched chain hydroxy acids, alanine, lactate, ketone bodies and several others. During the described treatment, the attention was focused on serum levels of BCKA's and BCAA's as the most important to be monitored by NMR in that the former seem to be directly responsible of neurotoxicity and should hopefully disappear from serum, while the latter must be prevented from dropping too much, since BCAA's are essential amino acids and hence must be under control during the therapy. It is worth noting,

however, that in serum, lactic and pyruvic acids and keton bodies were slightly increased, while alanine was often decreased; among the hydroxy acids, particularly 2-hydroxyisovaleric was often detectable in the 100μM range.

The utility of an NMR monitoring of serum in these respects is illustrated in Fig. 2 where it can be noted that after 20 days of BCAA's lacking diet aimed to achieve the disappearance of BCKA's from serum, normal levels of Val and decreased amounts of its catabolite KIV had promptly been reached, as well as a steady lowered level of KMV; conversely, the levels of Ile + Leu and of KIC were not significantly decreased. During diet A treatment, the thiamine therapy was also attempted, according to a routine protocol (Danner and Elsas, 1989).

These findings lead to identify the pathology as a non thiamine responsive classic MSUD, with leucine being the most persistently high BCAA; conse-

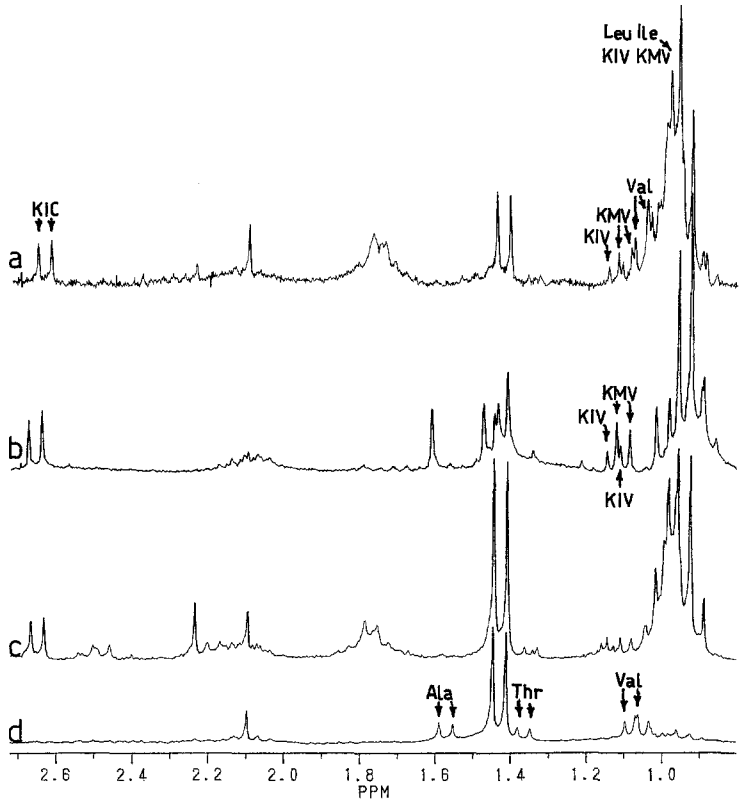

Fig. 1. Spectra of serum (**a,d**), urine (**b**) and CSF (**c**) of the patient. Spectra "a" and "b" were taken in the 16th day of life, before any treatment, and are the sum of 32 scans (4 min long), spectrum "c" was run during the 26th day, during diet A (1024 scans, 2 hours) and spectrum "d", by no means different from a normal one, in the 47th day (512 scans, 1 hour), during diet C, when normal serum contents of BCAA's were evidenced and BCKA's were not detected any further. Spectra "a" and "d" are in the same vertical scale to illustrate both the unambiguousness of diagnosis and the success of diet treatment. The arrows identify the peaks assigned to the indicated metabolites (see abbreviations)

quently, in the further diet therapy the Leu daily allowance was the lowest among BCAA's. From then on, the intake of BCAA's at the amounts of Leu, Ile and Val indicated in Table 1 was introduced in the diet. Under this new diet, Leu level finally decreased towards normal values, in good agreement with previous reports (Naughten et al., 1985; Shigematsu et al., 1983) while KIC level dropped (Shigematsu et al., 1983) below the sensitivity threshold of NMR.

It must be noted that a critical step in this diet treatment is the correct assessment of the daily allowances of BCAA's to be administered to the patient, in order to avoid BCKA accumulation while mantaining the normal serum levels of BCAA's, which are essential for a normal growth of the newborn. Although by some authors higher BCAA levels are considered acceptable (Shigematsu et al., 1983), it was decided to aim at keeping the serum level of such amino acids within the range described for normal newborn children (Leu + Ile 120 to 290 μM; Val 200 to 450 μM). As an example of the utility of NMR monitoring, after 9 days of diet B, involving a daily allowance of 315 mg of valine, the administered amount of the amino acid was decreased to 210 mg per day (diet C) as a consequence of the detection of increasing serum levels, above the normal range, (see Fig. 2).

Spectra of urine samples in the first day after diagnosis showed Ile + Leu 2450 μM, Val 350 μM, KIC 3750 μM, KIV 520 μM and KMV 1950 μM (Creatinine 0.56 mM). After 32 days (47th of life), a noticeable drop in BCAA's and BCKA's was obtained: Ile + Leu was 165 μM, Val 90 μM, KIC 100 μM, KIV about 20 μM while KMV was not detected (Creatinine 1.6 mM).

Table 1. Diet treatment

Diets A, B and C were all based on a mixture prepared in the following proportions:

"MSUD" (Milupa): BCAA-free amino acids	g 50
Sucrose	g 35
"Nŧdex" (Nestlè): Dextrose, maltose and sucrose	g 5
water	cc 100

To such mixture a low amount of natural protein containing modified milk formula and Leucine, Isoleucine and Valine as free amino acids were added in order to provide the following total (protein-bound + free) BCAA daily allowances:

Diet	Leu mg/day	Ile mg/day	Val mg/day
A	0	0	0
B	140	280	315
C	140	280	210

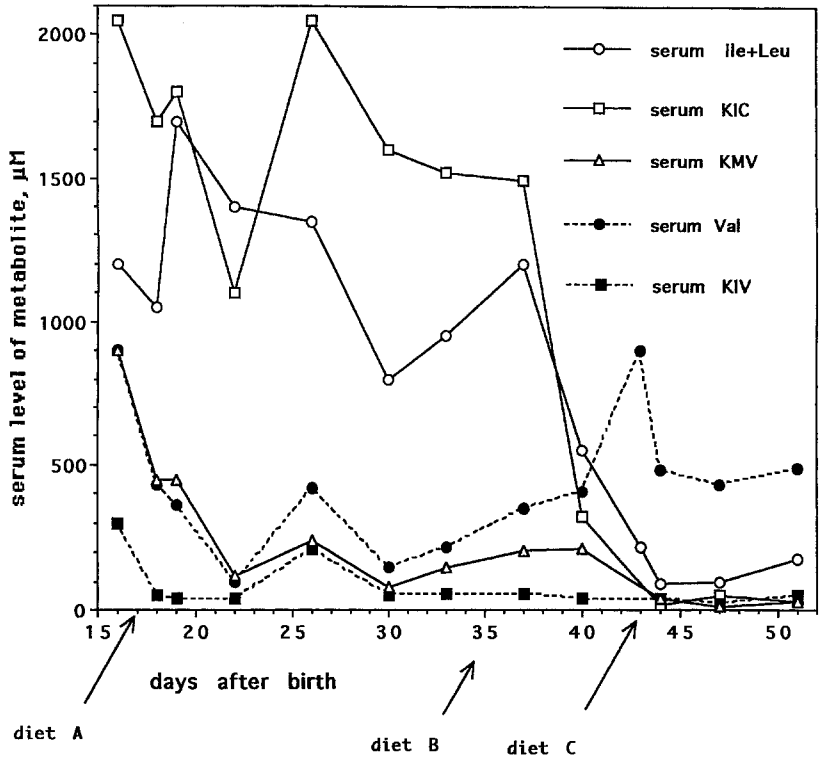

Fig. 2. Time dependent modifications of serum levels of BCAA's and BCKA's during diet treatment of the MSUD patient. Arrows indicate the first day of a diet treatment. Diets are as described in Table 1

Conclusions

The proposed NMR method has been proved to be suitable not only in diagnosis, but particularly in the follow up of diet treatment of MSUD. It gives, in a very short time all the necessary information in terms of metabolite estimates to allow a safe monitoring of the patient. To obtain otherwise the same set of data it would be necessary to have recourse to several different cumbersome and time consuming methods such as amino acid analysis, HPLC and gaschromatography-mass spectrometry.

References

Danner DJ, Elsas LJ II (1989) Disorders of BCAA and keto acid metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) Stanbury JB, Wyngaarden JB, Fredricksson DS (consulting eds) The metabolic basis of inherited diseases, vol 1. Mc Graw Hill, New York, pp 671–692

Elsas LJ, Ellerine NP, Klein PD (1993) Practical methods to estimate whole body leucine oxidation in maple syrup urine disease. Pediat Res 33: 445–450

Hoffer LJ, Taveroff A, Robitaille L, Mamer OA, Reimer ML (1993) Alpha keto- and alpha hydroxy branched chain acid interrelationships in normal humans. J Nutr 123: 1513–1521

Iles RA, Chalmers RA (1988) Nuclear magnetic resonance spectroscopy in the study of inborn errors of metabolism. Clin Sci 74: 1–10

Lehnert W, Hunkler D (1986) Possibilities of selective screening for inborn errors of metabolism using high resolution 1H-FT-NMR spectrometry. Eur J Pediatr 145: 260–266

Naughten ER, Saul IP, Roche G, Mullins C (1985) Early diagnosis and dietetic management in newborn with maple syrup urine disease. Birth to six weeks. J Inher Metab Dis 8 [Suppl] 2: 131–132

Pontoni G, Rotondo F, Dardo G, Cartenì-Farina M, Zappia V (1994) Proton magnetic resonance spectroscopy of biological fluids: a powerful tool in the diagnosis of inherited metabolic diseases. Eur J Clin Chem Clin Biochem 32: A7

Shigematsu Y, Kikuchi K, Momoi T, Sudo M, Kikawa Y, Nosaka K, Kuriyama M, Haruki S, Sanada K, Hamano N, Suzuki Y (1983) Organic acids and branched chain amino acids in body fluids before and after multiple exchange transfusions in maple syrup urine disease. J Inher Metab Dis 6: 183–189

Snyderman SE, Sansaricq C (1985) Newborn screening for maple syrup urine disease. J Pediatr 107: 259–261

Tofts PS, Wray S (1988) A critical assessment of methods of measuring metabolite concentration by NMR spectroscopy. NMR in Biomed 1: 1–10

Wevers RA, Engelke U, Heerschap A (1994) High resolution 1H-NMR spectroscopy of blood plasma for metabolic studies. Clin Chem 40: 1245–1250

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