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Effect of vitamin B12 derivatives on urinary excretion of methylmalonic acid in liver diseases*

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Abstract

1. Twenty.one patients with liver diseases were studied for their urinary mehylmalonic acid excretion after a valine load by means of an improved thin layer chromatography. 2. Methylmalonic acid positive cases were found in four out of the ten patients with cirrhosis of the liver, all four with cirrhosis and diabetes mellitus, and none with acute hepatitis of icteric phase. No apparent correlation was found between the methylmalonic acid excretion and the extent of hepatic damage. 3. A large amount of methylmalonic acid found in the case (S. I.) with cirrhosis of the liver and diabetes mellitus after the valine load was not corrected by cyanocobalamin but by DBCC, suggesting an impaired transformation from cyanocobalamin to DBCC. However, the nature of the impairment remains unknown.

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EFFECT OF VITAMIN B_{12} DERIVATIVES ON URINARY EXCRETION OF METHYLMALONIC ACID IN LIVER DISEASES

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Methylmalonic acid[1] is excreted in increased amount in the urine of patients with vitamin B_{12} deficiency (1, 2, 3, 4, 5). The coenzyme form[2] of vitamin B_{12} is required for the conversion of methylmalonyl-CoA to succinyl-CoA, and the appearance of DECC in human and animal livers has been also demonstrated (6). On the other hand, cyanocobalamin or hydroxocobalamin is converted into DBCC in rat and human livers (7), and dietary restriction of vitamin B_{12} results in a decrease of the hepatic coenzyme level (8). A possibility exists, therefore, that an impaired conversion of vitamin B_{12} into DBCC might cause the lowering of the liver coenzyme content, hence the excretion of MMA.

Recently, UKYO (9) reported patients with liver diseases who excreted MMA in urine after oral administration of valine which enhances MMA excretion in vitamin B_{12} deficiency (5, 10). The present study was undertaken to know in what liver diseases MMA is excreted in urine and what effects vitamin B_{12} derivatives have on the MMA excretion. The estimation of MMA in urine was carried out by a rapid thin layer chromatographic technique and its validity was also studied.

SUBJECTS AND METHODS

Twenty-four patients mostly of liver diseases (see Fig. 2) admitted to the Okayama University Hospital and its affiliated hospitals have been studied. The patients have not received parenteral administration of vitamin B_{12} derivatives and their oral administration, when they had been received, was discontinued at least for ten days before collection of urine for MMA assay.

Urinary MMA was estimated semiquantitatively by thin layer chromatography essentially as described by BASHIR and others[3] (11) except for the develop-

1. MMA: methylmalonic acid
2. DBCC: 5,6-dimethylbenzimidazolylcobamide coenzyme
3. In our preliminary experiments with their solvent system, a difficulty was found in separatipg MMA from hippuric acid, which is usually present in large quantities in urine. This difficulty could be eliminated by use of the system of DREIFUS and DUBE with minor modification;

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ing solution which was replaced by that of DREIFUS and DUBE (12) after a slight modification of the solvent system ; the ethanol concentration was reduced to 74 per cent (v/v) in order to increase the R_f value of MMA (Fig. 1). The quantitation of MMA concentration on the chromatogram was done according to UKYO and WAKISAKA (9). The recovery of added MMA (40 μg/1 ml urine) from normal urine was found to be 80 per cent, this value being used for correction. Since the lower limit of determination by the present method was 10 μg, for urine samples containing less than 10 μg MMA, an adequate amount of MMA was added to bring MMA content to a measurable range in the final application to the thin layer plate.

Succinate content in urine was determined by thin layer chromatography using the two kinds of developing solution as described before for the separation of MMA (11, 12).

Several metabolite contents in blood were estimated before and after administration of DBCC. Those included blood sugar (13), serum NEFA (14), blood

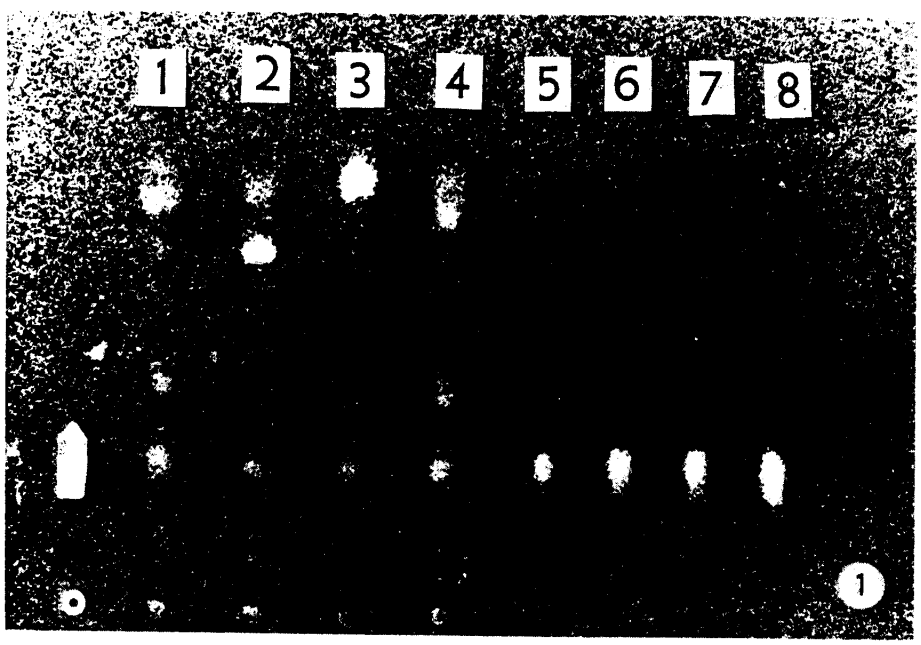

Fig. 1 A thin layer chromatogram showing BCG-positive spots of authentic MMA and ether extracts from urine samples 1, 2 and 4, urine from a MMA-excreting patient (Case S. I.); 3, internal standard (normal urine plus authentic MMA); 5, 6, 7 and 8, MMA standards, 20, 30, 40 and 50 g, respectively (R_f: 0.22) O: origin. Arrow indicates the direction of development. Succinic acid appears as a distinct comet-shaped spot ahead of methylmalonic acid. In order to avoid a possible effect of the presence of other urinary metabolites on R_f value of MMA, the identification of an equivocal spot was established by confirming the addictive effect of authentic MMA or by the response of the spot size to vitamin B_{12} administrations.

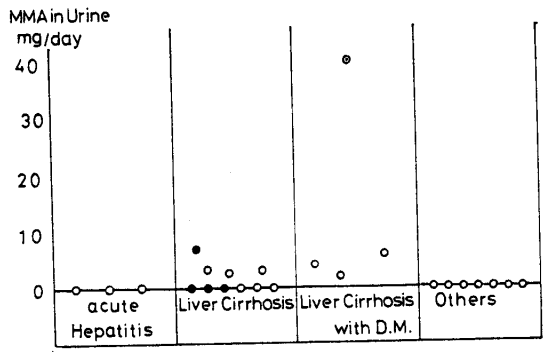

Fig. 2 Urinary MMA excretion in liver diseases after the valine load
Each circle represents one patient. The patients with acute hepatitis had jaundice at the time of this examination.
●: severe cases of cirrhosis of the liver, ⊙: Case S. I. Ohters included 2 diabetes mellitus, 2 chronic hepatitis, 1 Budd-Chiari syndrome, 1 primary hepatoma and 1 healthy adult.

pyruvate (15), lactate (16), citrate (17), and α-ketoglutarate (18). DBCC used for injection was the product of Yamanouchi Pharmaceutical. Co., Ltd (Tokyo). MMA was obtained from Sigma Chemical Company.

RESULTS

Urinary excretions of MMA after the valine load in liver diseases are shown in Fig. 2. No MMA was detected in the urine of any of the three patients with acute hapatitis. Among fourteen patients with cirrhosis of the liver, eighth showed positive MMA in urine. Severity of the disease as evidenced by jaundice, ascites, hyperammonemia, etc. was not always correlated with the positive result, while all four patients of cirrhosis were complicated with diabetes mellitus excreted MMA in various degrees. Other seven subjects including two diabetes with neuralgia, two chronic hepatitis, one Budd-Chiari syndrome, one primary hepatoma and one healthy adult developed negative result.

Further examinations in relation to vitamin B_{12} treatment were performed on one patient of liver cirrhosis with diabetes mellitus whose urinary MMA excretion was 42 mg in twenty-four hours after the valine load.

Case Presentation

S. I., a 58-year-old female, was admitted to the Okayama University Hospital on May 19, 1969. She had gradually noticed malaise and anorexia since three years before admission. Diagnoses of liver cirrhosis and diabetes mellitus were made and she had been treated with medicines untill the time

of hospitalization. Ten days before admission she developed an intermittent fever, expectoration and tender swelling of the left arm (phlegmon).

Physical findings revealed distended abdomen and enlarged splenic dullness, although liver and spleen were not palpated. Urinary sugar was positive while negative for ketone bodies. The hemoglobin concentration was 10.5 g/100 ml; erythrocytes, 3,880,000/mm³; the color index, 0.8; leucocytes, 14,700/mm³, with 85% neutrophils including 36% band form; and platelets, 135,000/mm³. The serum bilirubin was 0.85 mg direct and 1.49 mg total/100 ml; serum cholesterol, 70mg/100 ml; serum iron, 92γ/100 ml; fasting blood sugar, 127 mg/100 ml; SGOT and SGPT, 27 and 22 Karmen units, respectively; alkaline phosphatase, 3.3 Bessey units; the cobalt chloride reaction, R_6; CCF, three positive; TTT, 5 Maclagan units; ZnTT, 12 Kunkel units; and the total serum protein, 5.2g/100 ml; with 36.5% γ-globulin. The bromsulphthalein retention was 24.8% after 45 minutes. The other laboratory findings remained within normal limits.

One month's admission brought her marked recovery in general condition. On June 28 MMA excretion after the valine load was found to be 42 mg per day and its excretion rose to 105 mg even after intramuscular administration of a daily dose of 0.5 μg of cyanocobalamin for seven days. However, when the same dose of DBCC was given in the same way, MMA excretion fell to 39 mg per day and disappeared by 50 μg of DBCC. On September 18, MMA appeared in urine again inspite of daily oral administration of 1500 μg cyanocobalamin and came to undetectable by repeated treatment of 100 μg of DBCC (Fig. 3). When MMA excretion

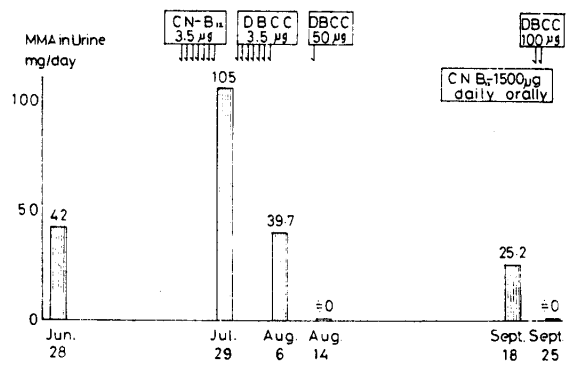

Fig. 3 Urinary MMA excretion during the course of vitamin B_{12} treatment
Each hatched bar and the number above it indicate the amount of MMA in 24-hour urine after the valine load. Each arrow represents a single daily injection of vitamin B_{12} and the total dose is given in the rectangle above.
CN-B_{12}: cyanocobalamin

decreased to nearly zero after 50 μg of DBCC injection, it was noted that succinate excretion in urine rose to a large quantity, while urinary valine excretion barely exceeded the normal level.

The effect of DBCC treatment on some blood metabolites in the valine load was studied and summarized in Fig. 4A and 4B. A sharp transient increase in pyruvate, but not in lactate and glucose following the valine load was found after DBCC treatment. The elevated blood citrate and NEFA decreased to nearly normal levels, while α-ketoglutarate level was high and unchanged.

Throughout the four months of her illness, she maintained erythrocyte counts of 380—400$\times 10^4$/mm^3, with hemoglobin levels of 10.5—11g/100 ml. The bone marrow was not examined.

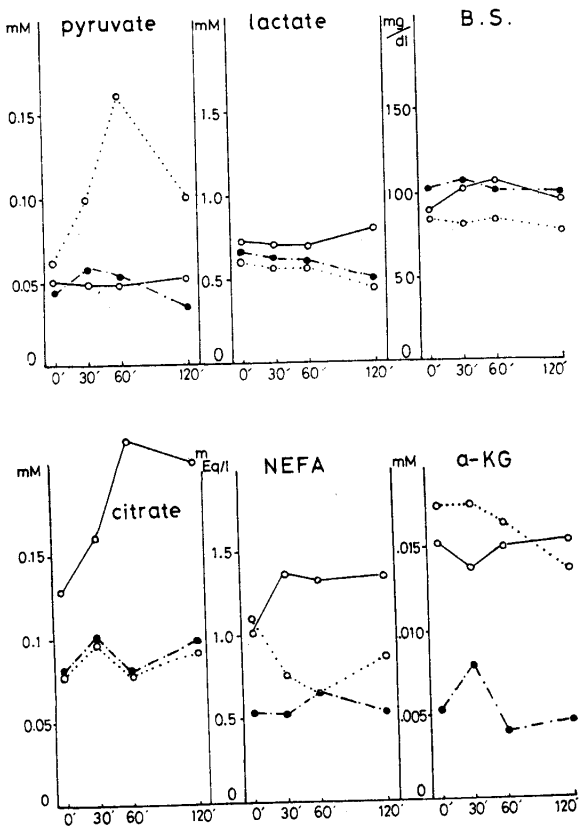

Fig. 4A and B The effect of cobamide coenzyme on blood metabolites in the valine load

○——○, before DBCC administration; ○···○, after DBCC administration

●-·-·●, normal male control without DBCC administration

DISCUSSION

UCHINO (8) reported that conversion from cyanocobalamin or hydroxocobalamin to coenzyme form of vitamin B_{12} occurred in normal rat liver and CCl_4 injuries resulted in a significant decrease in DBCC content in liver, indicating the disturbance of DBCC formation in some conditions with liver injury. A possibility exists that deficiency of cobamide coenzyme in tissues and urinary MMA excretion might occur in severe liver damage. This was extensively studied by UKYO and WAKISAKA (9) in liver diseases and CCl_4-injured rats, and they suggested the presence of an impaired conversion of vitamin B_{12} to DBCC in liver injuries in man as well as in experimental animals. The present clinical study confirmed their results by means of the improved thin layer chromatographic technique, which could eliminate overlapping of MMA and hippuric acids spots.

Clinical observations seen in Fig. 2 indicate that urinary MMA excretion after the valine load occurs not in the patients with acute hepatitis of icteric phase, but in those with more chronic liver damage. It is of interest that all four cases ccomplicated with diabetes responded positively for urinary MMA and in one of them (S. I,) the excraction of large amounts of MMA.

We could not find out a clear clinical difference between MMA positive and negative cases, on the point that MMA negative cases were noticed even among the patients with severe cirrhosis. In other words, there is no apparent correlation between the MMA excretion and the extent of hepatic damage. In the case of S. I. MMA excretion in urine was not corrected by cyanocobalamin but by DBCC administration. This is far from the evidence that not only the spot separated by the present MMA with thin layer chromatography is identified as MMA, but also the method excretion in this patient is due to the impaired transformation fromcyanocobalamin to DBCC. However, the nature of this impairment has not been clarified yet.

Recently MUDD, S. H. and his associates (19) reported an infant with abnormalities of both sulfur amino acids and methylmalonic acid metabolism and concluded as a failure to transform vitamin B_{12} into coenzymatically active derivatives. The urine of this patient consistently contained abnormally elevated homocystine, cystathionine, the mixed disulfide of homocysteine and cystein, and MMA. Methionine was not detected in urine.

The decrease in MMA excretion seen in the present case following DBCC treatment was associated with increased excretion of succinate,

enhanced formation of pyruvate after the valine load, and decreased blood citrate at zero time and NEFA levels. An increased pyruvate formation from amino acids in cirrhosis of the liver was reported by KIMURA (20). The elevated blood citrate and NEFA levels are known to be associated with gluconeogenic or ketogenic condition (21). A metabolic keto-acidosis was also observed in the methylmalonic aciduria, probably an inborn enzymatic defect in the conversion of methylmalonyl-CoA to succinyl-CoA (22, 23).

Accordingly, these evidences support the idea that the present case was improved by DBCC treatment not only in terms of MMA excretion but also in terms of latent ketotic and hindered gluconeogenic conditions.

SUMMARY

1. Twenty-one patients with liver diseases were studied for their urinary mehylmalonic acid excretion after a valine load by means of an improved thin layer chromatography.

2. Methylmalonic acid positive cases were found in four out of the ten patients with cirrhosis of the liver, all four with cirrhosis and diabetes mellitus, and none with acute hepatitis of icteric phase. No apparent correlation was found between the methylmalonic acid excretion and the extent of hepatic damage.

3. A large amount of methylmalonic acid found in the case (S. I.) with cirrhosis of the liver and diabetes mellitus after the valine load was not corrected by cyanocobalamin but by DBCC, suggesting an impaired transformation from cyanocobalamin to DBCC. However, the nature of the impairment remains unknown.

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