Studies on Methylmalonic Acid in Humans. II. Relationship between Concentrations in Serum and Urinary Excretion, and the Correlation between Serum Cobalamin and Accumulation of Methylmalonic Acid

Karsten Rasmussen, Lars Moelby, and Mogens Krogh Jensen

Methylmalonic acid (MMA) concentrations are increased in cobalamin (vitamin B_{12}) deficiency, but the relative diagnostic usefulness of determination of MMA in serum vs urine has not yet been assessed. We obtained urine collections and matched serum samples from 28 healthy volunteers and from 20 consecutive patients admitted for clinical and hematological evaluation because of low cobalamin concentrations in serum. Increased concentrations of MMA in serum were found in 12 patients, in all of whom a clinical diagnosis of cobalamin deficiency was established. By contrast, cobalamin deficiency was excluded in seven of the eight remaining patients, who all had normal MMA concentrations. Here we report that linear relationships exist between MMA concentrations in serum (investigated range: 0.05-34.2 μmol/L) and MMA concentrations in urine (r = 0.74), concentrations relative to creatinine (r = 0.98), and MMA excretion rates (r = 0.97) (P < 0.001 in each instance). Our data are consistent with glomerular filtration and passive reabsorption of MMA by the tubules. We demonstrate, for the first time, a negative correlation between concentrations of cobalamin and MMA in serum in clinical cobalamin deficiency (r = −0.69; P < 0.01; n = 12); when the values for MMA were log transformed, the correlation with cobalamin was much better (r = −0.84; P < 0.0005).

Additional Keyphrases: cobalamin status • metabolism

Determination of methylmalonic acid (MMA) in serum or urine for evaluating cobalamin deficiency is becoming an important diagnostic procedure in clinical chemistry, especially in patients with neurological disorders with few or no hematological abnormalities, or normal or only slightly depressed cobalamin concentrations in serum (1–3). Because the concentrations of MMA in urine are considerably higher than in serum, most studies have dealt with its measurement in the former (4, 5). However, the relationship of the MMA concentration in urine to that in serum is unknown, but it must be established before urinary data can be unambiguously interpreted.

As a result of newly developed analytical procedures, MMA can now be reliably measured in serum (6, 7). A significant advantage of the determination of MMA in serum as compared with its determination in urine is that the former is routinely collected in the process of evaluating patients for cobalamin deficiency, so serum specimens are usually available for additional studies in the clinical laboratory.

Surprisingly, the relative diagnostic usefulness of determination of MMA in serum vs urine has not yet been assessed (2). In 1985, Marcell et al. (6) found no significant correlation between concentrations of MMA in serum and urine from normal subjects. However, in 1986 Stabler et al. (1), studying sequential values of MMA in serum and urine from a patient with classic pernicious anemia, found evidence that measurement of MMA in serum and urine would correlate well with one another as diagnostic tests for cobalamin deficiency; they concluded that additional patients should be studied before this could be assessed with certainty.

The present work compares the concentrations of MMA in serum and its urinary excretion, and shows a highly significant positive correlation in normomethylmalonic-acidemia as well as in hypermethylmalonic-acidemia. We present data characterizing the renal handling of MMA and we report a significant negative correlation between concentrations of cobalamin and MMA in serum.

Specimens and Methods

Subjects

We obtained serum and matched 24-h urine collections from 28 overnight-fasted, healthy volunteers (14 men and 14 women, ages 22–86 y) and stored aliquot at −20 °C until they could be analyzed. Matched serum and urine specimens were collected from 20 patients (six men and 14 women, ages 26–82 y), consecutively selected on the basis of having cobalamin concentrations in serum below 100 pmol/L (normal range: 135–590 pmol/L), admitted for clinical and hematological evaluation at Aalborg Hospital, Denmark. In 15 of the patients, 18-h urine specimens were collected from 1300 to 0700 h, but it was not possible to obtain timed urine collections from five patients. All volunteers and patients had normal renal function (mean serum creatinine ± SD: 80 ± 11 and 77 ± 12 μmol/L, respectively).

Clinical diagnosis of cobalamin deficiency was made without knowledge of the MMA result (blinded test results), and MMA was measured without knowledge of clinical information (blinded clinical data). The criteria for clinical cobalamin deficiency were serum cobalamin concentration less than 100 pmol/L and one or both of the following: cobalamin absorption (Schilling) test result <10% and megaloblastic bone marrow morphology.

Determinations

MMA in serum and urine was determined by stable-isotope-dilution with solid-phase extraction of the samples (7). The total analytical imprecision of our method (SD) is 0.026, 0.025, and 2.1 μmol/L at concentrations in serum of 0.07, 0.30, and 40 μmol/L, respectively (7, 8). Similar figures are obtained for urine. For serum and urine, the normal reference intervals are 0.08 to 0.56 μmol/L and 0.58 to 3.56 mmol per mole of creatinine, respectively (7).

Received May 5, 1989; accepted August 8, 1989.
Creatinine in urine and in a serum sample obtained at the beginning of the urine collecting period was determined by the Technicon automated alkaline picrate method (9). We then calculated the fractional clearances of MMA relative to creatinine by standard formulae for renal clearance.

Serum cobalamin concentrations were determined with a radioisotope cobalamin/folate method from Amersham International, Bucks., U.K., involving denaturation of endogenous binding proteins at strongly alkaline pH and use of purified hog intrinsic factor as binding protein.

Statistical analysis. We used the coefficient of linear regression to establish the relative relationships between concentrations of MMA in serum and urine, and between concentrations of cobalamin and MMA in serum.

Results

Figures 1 and 2 illustrate the significant positive correlations in normo- and hypermethylmalonic-acidemia between concentration of MMA in serum and its urinary excretion, whether expressed as concentration of MMA relative to creatinine, substance concentration, or MMA excretion rate.

In normomethylmalonic-acidemia, a weak positive correlation was obtained between age and MMA concentration in serum ($r = 0.29; P < 0.05; n = 36$) and a highly significant positive correlation was found between the concentration of MMA relative to creatinine in urine and the urinary MMA excretion rate ($r = 0.99; P < 0.0005; n = 35$). No correlations were found between urine pH, which ranged from 5.5 to 7.3, and urinary excretion of MMA, or fractional MMA clearance relative to creatinine. Similarly, no correlations were found between diuresis, which ranged from 33 to 128 mL/h, and fractional MMA clearance relative to creatinine, but a very weak correlation ($r = 0.29; P < 0.10; n = 32$) was observed between diuresis and urinary MMA excretion rate. No correlation was found between concentrations of MMA, whether normal or increased, and fractional MMA clearance rate.

Fig. 1. Correlations of urinary MMA excretion rate (μmol/h), concentration of MMA in urine (μmol/L), and urinary excretion of MMA relative to creatinine (mmol/mol creatinine) with concentration of MMA in serum in normomethylmalonic-acidemia

Fig. 2. Correlations of urinary MMA excretion rate (μmol/h), concentration of MMA in urine (μmol/L), and urinary excretion of MMA relative to creatinine (mmol/mol creatinine) with concentration of MMA in serum in normo- and hypermethylmalonic-acidemia

Dashed lines indicate upper reference limits (7) for urinary excretion relative to creatinine ($≤ 3.56$ mmol/mol creatinine) and for concentration in serum ($≤ 0.56$ μmol/L).

Table 1 compares the data on the investigated subgroups. Increased concentrations of MMA in serum and urine were found in 12 patients, in all of whom a clinical diagnosis of cobalamin deficiency was established. By contrast, cobalamin deficiency was excluded in seven of the eight remaining patients, who all had normal values for MMA in serum. The diagnosis was uncertain in one patient with neurological abnormalities, in whom results for the Schilling test were abnormal (7%, normal ≥10%) but who had normal values for hemoglobin, mean cell volume, blood and bone-marrow smears, and for concentrations of MMA, folate, lactate dehydrogenase, iron, and transferrin in serum.

Due to the criterion for selection, all 20 patients had low concentrations of cobalamin in serum. In patients with
normomethylmalonic-acidemia, the values ranged from 64 to 86 pmol/L (mean 76 pmol/L). In patients with hypermethylmalonic-acidemia, the values ranged from 2 to 70 pmol/L (mean 40 pmol/L); only two of the 12 with hypermethylmalonic-acidemia had values (69 and 70 pmol/L) within the above range (64–86 pmol/L) observed in normomethylmalonic-acidemia.

In hypermethylmalonic-acidemia, a negative correlation existed between concentrations of cobalamin and MMA in serum (r = −0.69; P < 0.01; n = 12). In normomethylmalonic-acidemia, no significant correlation was found.

**Discussion**

It is evident from Figures 1 and 2 that a linear relationship exists between concentration of MMA in serum and in urine (and MMA concentration relative to creatinine, and MMA excretion rate). One consequence of practical importance is that determination of MMA in urine and serum may provide similar information on perturbations of MMA metabolism in cobalamin deficiency. However, as reported in the accompanying paper (8), in three healthy adults the urinary concentrations of MMA (in contrast to the urinary MMA excretion rates and the concentrations of MMA relative to creatinine), measured on multiple consecutive 3-h urine collections, did not correlate with concentrations of MMA in serum, undoubtedly because of variations in diuresis during the day. Furthermore, Marcell et al. (6), who determined MMA in serum and spot urine samples obtained from 50 blood donors, reported that neither serum MMA nor serum MMA per milligram of serum creatinine correlated significantly with urinary MMA or urinary MMA per milligram of urine creatinine. Together, these findings suggest that a complete 18-h to 24-h collection is necessary for valid estimation of urinary excretion of MMA and that the widespread use of determining the excretion of MMA in random (untimed) urine samples must be discouraged.

In clinical practice, however, 24-h collection of urine is difficult. Furthermore, there are other points in favor of testing serum. There is no need for an additional assay for creatinine, the dietary influences in serum in normal man are far less marked than in urine (8), and, as already mentioned, measurements can be done on serum that remains after determination of cobalamin.

There is no reason to believe that MMA as a small (molecular mass = 118 g/mol) and very polar dicarboxylic acid is bound to plasma proteins. Therefore, some information about the renal handling of MMA can be gained from our data. The fractional clearance ratio relative to creatinine is lower than 1, defining net reabsorption. There is no dependence of the fractional MMA clearance on the serum concentration, and the correlations from Figures 1 and 2 show no apparent maximal reabsorption by the tubules. The overall fractional MMA clearance ratio averaged 0.48 (SD = 0.23; n = 48). This value agrees reasonably well with those previously reported. Oberholzer et al. (10) studied a patient who had an extremely increased concentration of MMA in the plasma (155 μmol/L). From their data, a fractional MMA clearance ratio of 0.78 can be calculated. Marcell et al. (6) found a mean fractional MMA clearance ratio of 0.28 (range and SD were not reported) for spot urine samples from normal subjects. They argued that this observation supported their concept that most of the MMA in serum is metabolized via unknown pathways, as has been observed in experiments in which [methyl-14C]malonic acid was administered to rats via intracardial injections (apparently unpublished results, cited in (11)). However, the simplest explanation of a fractional clearance ratio lower than 1 is renal reabsorption, possibly by passive transport, because the urine-to-serum ratio for MMA was never less than 1 (see Figures 1 and 2), because the fractional MMA clearance was independent of concentration in serum, and because the urinary excretion rate varied with urine flow rate (although with weak correlation). Urine-to-serum gradients for acids can also be generated by pH gradients, but no correlation was found between urine pH and urinary excretion of MMA. Finally, MMA is not a candidate for the transport system for tricarboxylic acid cycle intermediates in renal brush borders because substrates for the dicarboxylate receptor must contain a four-carbon, non-branched backbone (12).

However, no data exist on concentrations of MMA in serum from patients with renal failure or other clinical situations where urine flow is decreased. Increased MMA concentrations would support the hypothesis that passive reabsorption is occurring. In a preliminary study we assayed 10 consecutive serum specimens from patients with increased values for serum creatinine (mean 365 μmol/L; range 171–508 μmol/L; normal range 55–110 μmol/L). The MMA concentrations were generally increased and ranged

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**Table 1. Mean (and Range) Concentrations of Methylmalonic Acid in the Investigated Subgroups**

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers</th>
<th>Normal serum MMA</th>
<th>Increased serum MMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>MMA in serum, μmol/L</td>
<td>0.16</td>
<td>0.21</td>
<td>5.71</td>
</tr>
<tr>
<td></td>
<td>(0.05–0.28)</td>
<td>(0.13–0.28)</td>
<td>(0.67–34.2)</td>
</tr>
<tr>
<td>MMA in urine mmol/mol creatinine</td>
<td>0.93</td>
<td>1.26</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td>(0.24–1.92)</td>
<td>(0.98–1.81)</td>
<td>(1.76–317)</td>
</tr>
<tr>
<td>MMA in urine μmol/L</td>
<td>6.15</td>
<td>6.95</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>(1.93–16.3)</td>
<td>(3.31–19.2)</td>
<td>(26.4–1169)</td>
</tr>
<tr>
<td>MMA in urine μmol/h</td>
<td>0.44</td>
<td>0.46*</td>
<td>14.7b</td>
</tr>
<tr>
<td></td>
<td>(0.15–1.22)</td>
<td>(0.37–0.55)</td>
<td>(1.20–7.74)</td>
</tr>
<tr>
<td>Fractional MMA clearance</td>
<td>0.45</td>
<td>0.41</td>
<td>0.61</td>
</tr>
<tr>
<td>relative to creatinine</td>
<td>(0.12–1.18)</td>
<td>(0.20–0.62)</td>
<td>(0.23–1.11)</td>
</tr>
</tbody>
</table>

*Timed urine collections were obtained from only four patients. b Timed urine collections were obtained from 11 of the patients.
from 0.12 to 1.42 μmol/L (mean 0.53 μmol/L) compared with 0.05–0.28 μmol/L (mean 0.16 μmol/L) observed in the 28 healthy volunteers in the present study. However, there was no significant relationship between concentrations of creatinine and MMA (r = 0.28; P > 0.25).

In 1973, Chanarin et al. (13) reported a negative correlation (r = -0.41; P not stated; n = 30) of MMA excretion in 24-h urine collection with the serum cobalamin concentration (microbiological assay) in clinical cobalamin deficiency. Marcelli et al. (6) found a weak negative correlation with the concentration of MMA in serum (r = -0.25; P = 0.08; n = 50) in normal subjects (the serum cobalamin assay was not stated). Surprisingly, Stabler et al. (1) reported no correlation in 73 patients with clinically confirmed cobalamin deficiency (r = -0.12 for microbiologic assays and -0.09 for radioassays; P >0.40 in each instance). We were able to demonstrate a good, significant correlation between the cobalamin assay and determination of MMA in serum from the 12 patients with hypermethylmalonic-acidemia (r = -0.69; P<0.01). In normomethylmalonic-acidemia, no significant correlation was found. Because the normal reference interval for concentrations of MMA in serum is log-normally distributed (6, 7), we calculated the correlations between cobalamin concentrations and the logarithm of the individual values for MMA in serum and obtained much better correlation in hypermethylmalonic-acidemia (r = -0.84; P <0.0005; n = 12), but still no convincing correlation was obtained in normomethylmalonic-acidemia (P >0.25). The present work, however, was not designed to assess the diagnostic sensitivity and specificity of serum cobalamin assays. Further studies to elucidate this problem are contemplated.

The present study shows that determination of MMA in serum appropriately detected all cobalamin-deficient patients. If our results are supported by additional clinical research, assay for serum MMA may indeed become the reference standard test to establish the diagnosis of tissue cobalamin deficiency.

This work was supported in part by a grant from The Institute of Experimental Clinical Research, Aarhus University, Denmark. We thank Jette Jensen for skillful technical assistance.

References