Cystathionine Excess in Children with Hepatic Cancer

Lawrence Helson, Robert H. F. Peterson, and Morton K. Schwartz

SUMMARY

The 24-hr urinary excretion of cystathionine was determined by automated chromatography in five children with clinically apparent liver cancer (hepatoblastoma or hepatocellular carcinoma). Cystathionine was also detected by thin-layer chromatography in metastatic tumor tissue of two patients. Three patients had increased cystathioninuria at the time of diagnosis and two developed it during the course of their disease. Urinary excretion levels decreased following effective therapy and increased with recurrence of tumor in one patient. The lack of correlation between elevated urinary cystathionine excretion and the presence of small amounts of tumor and its nonspecificity for hepatic cancer limits its potential as a screening method.

INTRODUCTION

Secondary cystathioninuria in a male infant with hepatoblastoma was first reported in 1965 (6). Since then 6 male children with hepatoblastoma and cystathioninuria have been described (4, 14, 16, 23). In extending these observations, we have examined the 24-hr excretion of cystathionine in the urines of an additional 5 children with hepatic neoplasms.

MATERIALS AND METHODS

Between 1971 and 1972, 5 children with hepatic neoplasms were referred to Memorial Hospital for treatment. Histological diagnoses were based upon the criteria and classification set forth by Ishak and Glunz (12). As part of their initial evaluation, and at intervals during their disease course, these children had urinary amino acid screens including cystathionine. The 24-hr urine collections were acidified with 6 M HCl to pH 3.0 and refrigerated until the assays were performed. Aliquots were assayed for creatinine; cystathionine was determined by automated chromatography in five children with clinically apparent liver cancer (hepatoblastoma or hepatocellular carcinoma). Cystathionine was also detected by thin-layer chromatography in metastatic tumor tissue of two patients. Three patients had increased cystathioninuria at the time of diagnosis and two developed it during the course of their disease. Urinary excretion levels decreased following effective therapy and increased with recurrence of tumor in one patient. The lack of correlation between elevated urinary cystathionine excretion and the presence of small amounts of tumor and its nonspecificity for hepatic cancer limits its potential as a screening method.

RESULTS

Levels of urinary cystathionine varied in individual patients during the course of their disease. Abnormally elevated levels were detected in 3 patients, at the time of diagnosis and in 2 patients during the course of their disease. These data are shown, along with serum AFP determinations, in Table 1. In both Patients T. B. and M. M., the concentration of cystathionine extracted from metastatic implants was estimated to be 25 mg/100 g wet tissue.

DISCUSSION

The urine and tissue cystathionine values reported in the literature for patients with hepatoblastoma and neuroblastoma and in children without tumor and listed in Table 2. These values were determined in urine specimens from 6 healthy children. Blood samples from patients taken during the time of the urine collection were assayed for AFP (N = 0) by counterimmunoelectrophoresis with Kallestead antisera (Kallestead Laboratories, Minneapolis, Minn.) to AFP.

Detection of cystathionine in tumor from Patients T. B. and M. M. was accomplished by homogenization of 0.5 g tissue at room temperature in 0.5 ml of trichloroacetic acid (50 g/liter). After centrifugation, amino acids in the supernatant were identified with 1-directional thin-layer chromatography (20). Two-µl aliquots of solution containing 1.0, 0.5, and 0.125 µg cystathionine per µl were cochromatographed with the 2 µl of tissue extract on Avicel (microcrystalline cellulose) plates (Analtech Inc., Newark, Del.). The following solvent systems were used: A, 1-butanol:acetone:water:diethylamine (14:14:7:2:8); B, 1-butanol:acetic acid:water (12:3:5); C, phenol:water:ammonia (160:40:1). The average run was 2 hr, and after air drying for 1 hr the plates were developed with 0.1% ninhydrin spray (1 g/liter). Cystathionine standard was run with each plate because of the variability of cystathionine Rf values in the individual solvent systems: A, 0.16 to 0.25; B, 0.14 to 0.20; C, 0.32 to 0.34. Previous study with these solvent systems indicated that cystathionine could easily be separated from phosphoethanolamine and cysteine, both of which flow near but not with cystathionine. Quantitative estimations were based upon comparison with relative intensities of known reference standard compounds.

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The data of our study corroborate previous reports indicating that elevated levels of cystathionine may be found in the urine and tumor tissues of patients with hepatoblastoma. Urinary excretion levels of patients in this study were 5- to 13-fold lower than those reported by other investigators when cystathioninuria was expressed on a mg/g creatinine basis (Tables 1 and 2). This may be due to low creatinine excretion values; an explanation offered by Voute and Wadman (23) for their patient’s relatively high cystathionine/creatinine value. Nevertheless, the cystathionine excretion levels of these cancer patients are abnormal when compared to values previously reported for controls (3, 5, 15, 18, 21, 23), neuroblastoma patients who responded to treatment (9), and contemporary controls (Table 1).

In contrast with previous reports (Table 2) both females and patients with histological diagnoses other than hepatoblastoma, i.e., hepatocellular carcinoma, may have abnormal excretion levels. Voute and Wadman (23) did not document

Table 1
Quantitative urinary cystathionine in patients with liver neoplasms and 6 normal controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Creatinine (mg/g)</th>
<th>24-hr total (mg)</th>
<th>AFP</th>
<th>Clinical status at time of study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.4</td>
<td></td>
<td></td>
<td>7.6</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>7</td>
<td></td>
<td></td>
<td>0.26</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>8</td>
<td></td>
<td></td>
<td>0.39</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>13</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. M.</td>
<td>Hepatoblastoma mixed epithelial and mesenchymal type</td>
<td>F</td>
<td>0.38</td>
<td>38.0</td>
<td>2.4</td>
<td></td>
<td>1 wk after biopsy. Estimated 100 g tumor present.</td>
</tr>
<tr>
<td>T. B.</td>
<td>Hepatoblastoma epithelial type</td>
<td>M</td>
<td>8</td>
<td>330</td>
<td>20</td>
<td></td>
<td>Progressive cachexia and tumor growth.</td>
</tr>
<tr>
<td>B. B.</td>
<td>Hepatoblastoma mixed epithelial and mesenchymal</td>
<td>M</td>
<td>12</td>
<td>75.3</td>
<td>8.0</td>
<td></td>
<td>1 wk after percutaneous biopsy of massive tumor.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>After partial resection 3000 rads, and 2 wk chemotherapy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F3TDR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.1</td>
<td>8.9</td>
<td></td>
<td>After 2 mo. of F3TDR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>36.0</td>
<td>36.2</td>
<td></td>
<td>Evidence of pulmonary metastases</td>
</tr>
<tr>
<td>M. M.</td>
<td>Hepatocellular carcinoma</td>
<td>F</td>
<td>13</td>
<td>4.2</td>
<td>18.6</td>
<td></td>
<td>1 yr after right lobectomy, 2000 rads to the tumor site, and 2 mo. of F3TDR. No gross tumor evident.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
<td></td>
<td>11 mo. later. 150-g tumor found on abdominal exploration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.3</td>
<td></td>
<td>After 6 mo. Additional radiotherapy to metastatic sites, and other chemotherapy.</td>
</tr>
<tr>
<td>M. R.</td>
<td>Hepatocellular carcinoma</td>
<td>F</td>
<td>15</td>
<td>13.5</td>
<td>13.7</td>
<td></td>
<td>1 mo. after biopsy of massive tumor.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.5</td>
<td>13.1</td>
<td></td>
<td>After 3000 rads to the tumor site, and 2 mo. of F3TDR, the tumor mass remained unchanged.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64.0</td>
<td></td>
<td>Progressive tumor growth.</td>
</tr>
</tbody>
</table>

*F3TDR, trifluoro-2'-deoxyuridine (10).*
but alluded to the fact that adult patients with hepatic cancer may excrete elevated amounts of cystathionine.

Although the total number of patients studied is small, these data suggest that elevated urinary cystathionine levels are present in a variety of histological types of liver neoplasms. Elevated levels have also been found in patients with metastatic neuroblastoma and some other tumors (9). Its potential as a cancer screening test is limited by an apparent lack of correlation in excretion when compared to tumor volume. For example, Patient T. B. in the presence of normal urinary cystathionine excretion levels, had a large tumor (estimated to be 400 to 500 g) involving his liver and mesentry. At a later date, when his tumor mass was even more enlarged, his cystathioninuria became abnormally elevated. Multiple tumor sites had developed (ca. 150 g) in Patient M. M. by the time elevated cystathionine levels were detected. Following radiation therapy and chemotherapy her excretion levels then decreased in the presence of tumor progression. The other 3 patients with elevated cystathionine excretion values had massive disease at diagnosis. Geiser et al. (4) reported 3 patients with hepatoblastoma and 2 patients with hepatocellular carcinoma with negative excretion studies. The extent of disease in these patients and the relationship between assay and previous chemotherapy were not described. In the serial study of Patient B. B., decrease to zero cystathionine excretion followed surgical ablation, radiation therapy and systemic chemotherapy, only to return to elevated levels along with other abnormal serum chemistries and pulmonary metastases. The analytical chemical methods for detection in tissue are sensitive to 0.25 μg of cystathionine and permit the use of the assay in estimating the concentration of cystathionine in tissue following chemotherapy or radiotherapy. The utility of urinary determinations in gauging the course of the disease and effects of therapy is limited by the fact that tumor tissue may be present even in the absence of abnormal cystathionine excretion.

The detection of elevated amounts of cystathionine in tumor tissue of Patients T. B. and M. M. in this study and in 2 published studies (4, 23) inculpate the malignant cell as a specific source. The cystathioninuria in these patients can be attributed to increased tumor cell synthesis or decreased breakdown within the tumor cell or other tissues. A similar origin has been considered for the cystathioninuria found in patients with metastatic neuroblastoma (5). It has been demonstrated in humans that the concentration of cystathionine in the fetal liver is higher than in mature liver, and the enzyme cystathionase, absent in fetal liver, is present in the normal adult liver (19).

### Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>Ref.</th>
<th>Author</th>
<th>Subjects studied</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Urine Creatinine (mg/g)</th>
<th>Liver tissue mg/100 g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24-hr total</td>
<td></td>
</tr>
<tr>
<td>1965</td>
<td>6</td>
<td>Gjessing and Mauritzen</td>
<td>1</td>
<td>M</td>
<td>1.0</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>1967</td>
<td>14</td>
<td>Lieberman et al.</td>
<td>3</td>
<td>M</td>
<td>0.7</td>
<td>50</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>3.0</td>
<td>140</td>
<td>160</td>
</tr>
<tr>
<td>1968</td>
<td>16</td>
<td>Raine</td>
<td>1</td>
<td>M</td>
<td>1.6</td>
<td>Elevated</td>
<td></td>
</tr>
<tr>
<td>1968</td>
<td>23</td>
<td>Voute and Wadman</td>
<td>1</td>
<td>M</td>
<td>0.8</td>
<td>1238</td>
<td>22.3</td>
</tr>
<tr>
<td>1970</td>
<td>4</td>
<td>Geiser et al.</td>
<td>1</td>
<td>M</td>
<td>1.1</td>
<td>537</td>
<td>68.8</td>
</tr>
<tr>
<td>1971</td>
<td>10</td>
<td>Helson et al.</td>
<td>11</td>
<td></td>
<td>1.6–19</td>
<td>0–6.56</td>
<td>0–3.78</td>
</tr>
<tr>
<td>1963</td>
<td>5</td>
<td>Gjessing</td>
<td>10</td>
<td></td>
<td>0.2–10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>18</td>
<td>Scott et al.</td>
<td>7</td>
<td></td>
<td>2.0</td>
<td>0.022</td>
<td>Normal 0.7</td>
</tr>
<tr>
<td>1971</td>
<td>15</td>
<td>Lines and Waisman</td>
<td>7</td>
<td></td>
<td>5.85 (mean)</td>
<td>2.12</td>
<td>Liver 0.8</td>
</tr>
<tr>
<td>1958</td>
<td>22</td>
<td>Tallan et al.</td>
<td>Adult</td>
<td></td>
<td>0–5.85</td>
<td>0–2.12</td>
<td>Liver 1–20.6</td>
</tr>
<tr>
<td>1965</td>
<td>3</td>
<td>Brenton et al.</td>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td>Liver 2.6–3.5</td>
</tr>
<tr>
<td>1970</td>
<td>21</td>
<td>Sturman et al.</td>
<td>Newborn (premature)</td>
<td>1</td>
<td></td>
<td></td>
<td>Liver 2.0–3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Newborn (FT)</td>
<td>9</td>
<td></td>
<td></td>
<td>Liver 7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5–Adult</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>
progressive disease, has been described previously (2) and is
illustrated by Patient T. B. in whom increase in tumor mass
was associated with a conversion to positive AFP and elevated
urinary cystathionine levels. Not all patients with tumors
behave in such a fashion. In Patients L. M. and M. M.,
cystathionine excretion was abnormal in the presence of
undetectable AFP; however, the use of a more sensitive assay
for AFP may have detected levels above normal that were not
detected by the counterimmunoelectrophoretic method.

Both the presence of cystathioninuria in non-tumor-bearing
subjects with liver damage secondary to galactosemia, familial
cirrhosis, or glycogen storage disease (19) and the increased
AFP in patients with ovarian or testicular embryomas and
nonneoplastic liver disease (17) indicate that the biochemical
fetal characteristics may occur following a variety of patho-
physiological changes in normal and malignant tissues and may
be altered by treatment with radiotherapy and chemotherapy
agents.

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