DHP and β-UP could be detected in a variety of other tissues, especially in bronchus. In cerebellum, only the activity of DPD and DHP could be detected indicating that adult human brain cells are not able to synthesize β-alanine via the catabolism of uracil. In this respect, it should be noted that partial β-UP cDNAs have been detected in human EST libraries of infant brain (8).

Acknowledgements

We thank dr. C. van Noesel and prof. dr. D. Troost of the department of Pathology of the Academic Medical Center for providing the tissue samples.

Literature


Analysis of pyrimidine synthesis de novo intermediates in urine and dried urine filter-paper strips with HPLC-electrospray tandem mass spectrometry

A.B.P. van KUILENBURG1, H. van LENTHE1, M. LÖFFLER2 and A.H. van GENNIP3

Pyrimidine nucleotides are essential for a vast number of biological processes such as the synthesis of RNA, DNA, phospholipids, glycogen and the sialylation and glycosylation of proteins. Pyrimidines can be synthesized de novo in mammalian cells through a multistep process and via the salvage of the pyrimidine nucleosides uridine and cytidine. Proliferating cells usually require a functional pyrimidine de novo pathway to sustain their increased demand for nucleotides. Therefore, a number of inhibitors have been developed against enzymes of the pyrimidine de novo pathway, such as dihydroorotate dehydrogenase, which show potent antiproliferating effects in tumor cells or proliferating T-lymphocytes. Pathological conditions, such as a deficiency of UMP synthase or a urea-cycle defect can result in altered excretion of metabolites of the pyrimidine de novo pathway (1, 2).

Patients with a deficiency of UMP synthetase excrete excessive amounts of orotate in their urine. In contrast, patients suffering from a urea cycle disorder can, in addition to orotate, also excrete strongly elevated levels of orotidine, uridine and uracil (2). We have developed a fast and sensitive method, using HPLC-tandem mass spectrometry, allowing the detection of all pyrimidine de novo metabolites within a single analytical run of 14 min, using urine or urine-soaked filter paper strips (3).

Materials and Methods

Urine samples were centrifuged and 10 µl of the IS and 2 µl of 25% (v/v) HCOOH was added to 100 µl of clear urine. After centrifugation, 50 µl of this urine was injected into the HPLC-MS/MS system. Urine (200 µl) was added to filter paper strips, dried at room temperature and 20 µl of the IS was deposited on the strip. After drying, the strip was extracted with 1.5 ml of 75% (v/v) methanol, dried at 40 °C and subsequently dissolved in 200 µl of 50 mM HCOOH (pH 2.6) and sonificated for 5 min. After centrifugation, 50 µl of the clear extract was injected into the HPLC-MS-MS system.
The metabolites were separated on a Phenomenex Aqua analytical column (250 x 4.6 mm, 5 µm particle size), protected by a guard column (SecurityGuard C18 ODS; 4 x 3.0 mm; Phenomenex). Solvent A consisted of 50 mM HCOOH (pH 2.6) and solvent B consisted of a mixture of 50 mM HCOOH (pH 2.6) and methanol (1:1 by volume). The eluent from 4.2 to 8 min was introduced into the mass spectrometer. A Quattro II tandem mass spectrometer (Micromass) was used in the negative Electrospray ionization (ESI) mode and nitrogen was used as the nebulizing gas. Multiple-reaction monitoring was used to detect the metabolites by the specific m/z transition of precursor ion to fragment (3).

Results and Discussion

Urine or urine-soaked filter-paper strips were used to measure N-carbamyl-aspartate, dihydroorotate, orotate, orotidine, uridine and uracil. Reversed-phase HPLC was combined with electrospray ionization tandem mass spectrometry and detection was performed by multiple-reaction monitoring. Stable-isotope-labeled reference compounds were used as internal standards. All pyrimidine de novo metabolites and their degradation products were measured within a single analytical run of 14 min with the lower limit of detection ranging from 0.4 µM to 3 µM. The intra-assay variation and inter-assay variation of urine with added

<table>
<thead>
<tr>
<th>Patients</th>
<th>Enzyme defect</th>
<th>N-C-Aspartate</th>
<th>Dihydroorotate</th>
<th>Orotate</th>
<th>Orotidine</th>
<th>Uridine</th>
<th>Uracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ornithine transcarbamylase</td>
<td>15</td>
<td>n.d.</td>
<td>155</td>
<td>3.0</td>
<td>2.6</td>
<td>288</td>
</tr>
<tr>
<td>2</td>
<td>Ornithine transcarbamylase</td>
<td>34</td>
<td>0.6</td>
<td>823</td>
<td>102</td>
<td>1.5</td>
<td>45</td>
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<tr>
<td>3</td>
<td>Ornithine transcarbamylase</td>
<td>21</td>
<td>n.d.</td>
<td>263</td>
<td>2.0</td>
<td>46</td>
<td>231</td>
</tr>
<tr>
<td>4</td>
<td>Argininosuccinase</td>
<td>53</td>
<td>3.8</td>
<td>235</td>
<td>3.2</td>
<td>3.3</td>
<td>85</td>
</tr>
<tr>
<td>Controls (n = 155)</td>
<td></td>
<td>36</td>
<td>4.7</td>
<td>330</td>
<td>4.1</td>
<td>5.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>0.8 ± 0.7</td>
<td>0.01 ± 0.07</td>
<td>1.2 ± 0.9</td>
<td>1.4 ± 1.0</td>
<td>0.4 ± 0.7</td>
<td>7.9 ± 6.0</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>n.d.-3.8</td>
<td>n.d.-0.5</td>
<td>n.d.-6.2</td>
<td>n.d.-4.8</td>
<td>n.d.-3.5</td>
<td>n.d.-30</td>
</tr>
</tbody>
</table>

**Figure 1.** Pyrimidine *de novo* pathway. ①, carbamylphosphate synthetase; ②, aspartate transcarbamylase; ③, dihydorrotase; ④, CAD; ⑤, dihydroorotate dehydrogenase; ⑥, orotase phosphoribosyltransferase; ⑦, orotidine 5'-monophosphate decarboxylase; ⑧, UMP synthase; ⑨, orotidine 5'-monophosphate phosphohydrolase; ⑩, pyrimidine 5' nucleotidase; ⑪, uridine kinase; ⑫, uridine phosphorylase.
5-fluorouracil (5FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of cancers of the gastrointestinal tract, breast, head and neck. To exert its cytotoxic effect against cancer, 5FU must first be anabolized to the nucleotide level. Opposing the activation of 5FU to the level of fluoropyrimidine nucleotides are the enzymes of the pyrimidine degradation pathway. Dihydropyrimidine dehydrogenase (DPD) catalyzes the conversion of 5FU to fluoro-5,6-dihydrouracil which is the initial and rate-limiting step in the catabolism of 5FU. A relationship between the 5FU dose intensity and the therapeutic response, as well as toxicity, has been noted. Patients with a DPD deficiency are unable to degrade 5FU and these patients are at risk of developing severe toxicity after the administration of 5FU (1, 2). Therapeutic drug monitoring of the 5FU levels in plasma requires the fast and unambiguous identification and quantification of 5FU. In this study, we describe a fast and specific method to measure 5FU in plasma with HPLC tandem-mass spectrometry.

Materials and Methods
Plasma samples were obtained from colorectal patients receiving bolus administration of 5FU (425 mg/m²) and folinic acid (20 mg/m²). 30 µl of the internal standard (1,3-15N2-5FU) was added to 300 µl of plasma and centrifuged over a Microcon YM-30 filter to remove protein. 2 µl of 25% (w/v) HCOOH was added to 70 µl of the deproteinized plasma sample and 50 µl was injected into the HPLC-MS/MS method.

Figure 1. Comparison between the HPLC-MS/MS method and a HPLC-UV method. R² = 0.98, y = 0.98 x.

Determination of 5-fluorouracil in plasma with HPLC-tandem mass spectrometry
A.B.P. van KUILENBURG1, H. van LENTHE1, J. G. MARING2 and A. H. van GENNIP3

5-fluorouracil (5FU) remains one of the most frequently prescribed chemotherapeutic drugs for the treatment of cancers of the gastrointestinal tract, breast, head and neck. To exert its cytotoxic effect against cancer, 5FU must first be anabolized to the nucleotide level. Opposing the activation of 5FU to the level of fluoropyrimidine nucleotides are the enzymes of the pyrimidine degradation pathway. Dihydropyrimidine dehydrogenase (DPD) catalyzes the conversion of 5FU to fluoro-5,6-dihydrouracil which is the initial and rate-limiting step in the catabolism of 5FU. A relationship between the 5FU dose intensity and the therapeutic response, as well as toxicity, has been noted. Patients with a DPD deficiency are unable to degrade 5FU and these patients are at risk of developing severe toxicity after the administration of 5FU (1, 2). Therapeutic drug monitoring of the 5FU levels in plasma requires the fast and unambiguous identification and quantification of 5FU. In this study, we describe a fast and specific method to measure 5FU in plasma with HPLC tandem-mass spectrometry.

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Literature