
CLIN. CHEM. 35/5, 848–851 (1989)

A Simplified and Rapid Test for Acetylator Phenotyping by Use of the Peak Height Ratio of Two Urinary Caffeine Metabolites

Adnan El-Yazigi,1 Kutalba Chaleby,2 and Cazemiro R. Martin1

We describe a simplified liquid-chromatographic test in which acetylator phenotype is determined by measuring the peak height ratio of two urinary caffeine metabolites, 5-acetylamin o-6-formylamino-3-methyluracil and 1-methylxanthine. We applied this test to determine the acetylator phenotypes of 52 subjects who regularly drink coffee, tea, or caffeinated beverages. Also, we determined the acetylator phenotypes of these subjects according to a well-established sulfasalazine test, which yielded identical results. We established the reproducibility of this test by determining acetylator phenotypes of 10 additional subjects on two different days separated by a period of two to five weeks. Of the 52 subjects examined by both tests, 40 (76.9%) were classified as slow acetylat ors, which agrees well with the percentage reported elsewhere for 297 similar subjects from the Saudi population.

N-Acetyl transferase is an enzyme responsible for bio- transformation of several clinically important drugs, including procainamide, isoniazid, phenelzine, dapsone, hydralazine, and sulfonamides. The production of this enzyme in the liver and gastrointestinal mucosa is genetically controlled and hence the acetylation capacity of individuals within a population is subject to polymorphism with a bimodal or trimodal distribution pattern. Thus, a subject is classified as either a slow or rapid acetylator, with the latter being homozygote or heterozygote for this dominant character (1). In addition to its marked benefits in therapeutics, acetylator status has been linked to several disease states such as bladder cancer, diabetes mellitus, systemic lupus erythematosus, and Gilbert’s syndrome (2).

Several screening tests have been used for determining acetylator phenotype. These tests are based on the use of one of the above-mentioned drugs. Isoniazid has been the agent traditionally used (3) for these studies, but it has a complex metabolism and requires serial blood samples, which makes it inconvenient for routine, widespread use. Other commonly used tests involve a sulfonamide such as sulfadimidine, sulfapyridine, sulfasalazine, or sulfamethazine. These methods are based on the classical Bratton–Marshall procedure (4), and require the administration of a single oral dose of the drug and collection of multiple samples (5) or a single sample (6–9) of serum or urine. Dapsone was also used for acetylator phenotyping, a single sample of plasma being collected 2–72 h after administration of a single dose of this drug and the concentration ratio of monoacetildapsone to dapsone determined by high-performance liquid chromatography (10, 11). Similarly, the concentration ratio of N-acetylprocainamide to procainamide in plasma was used to determine the acetylator phenotype, and the results obtained were similar to those acquired with dapsone (12).

Recently, Grant et al. (13) demonstrated that the production of 5-acetylamin o-6-formylamino-3-methyluracil (AFMU), a caffeine metabolite, is mediated by N-acetyltransferase and that the molar ratio of AFMU to 1-methylxanthine (1MX), another metabolite of caffeine, in urine collected 2–6 h after consumption of a caffeinated beverage (coffee, tea, or soft drink) can be used for a large-scale assessment of acetylator phenotype (14). Because of the ubiquitous use and relative safety of caffeine and the ease and simplicity of the protocol used, this test may offer some advantages over other methods for population studies (2).

In this report, we further simplify the test by using the peak height ratio of AFMU/1MX in lieu of molar concentration ratio of these metabolites for determination of the acetylator status. This allows elimination of the internal standard and calibration curves that would be needed to estimate the concentrations of these metabolites. We compared the results obtained by this test with those obtained.

1 Biological and Medical Research Department and 2 the Department of Medicine, King Faisal Specialist Hospital & Research Centre, Riyadh 11211, Saudi Arabia.

Received December 28, 1988; accepted January 31, 1989.

with a sulfasalazine method based on a simple Bratton–Marshall procedure for which no special equipment is needed (9).

**Materials and Methods**

**Subjects**

We included in this study 62 subjects, primarily from Riyadh and its surrounding towns in the central region of Saudi Arabia. These subjects, whose ages ranged from 17 to 70 years, were all seen at the Psychiatry Clinic, King Faisal Specialist Hospital and Research Center, for different psychiatric problems such as social phobia and manic depression. They all had normal values for serum urea nitrogen, creatinine, uric acid, glucose, total bilirubin, alkaline phosphatase, and aspartate and alanine aminotransferases. Urinalysis also yielded normal results. We assessed the acetylator phenotypes of 52 of these subjects by the described caffeine-metabolites test and also according to a sulfasalazine method as described by Schroder (9). The remaining subjects were included to examine the reproducibility of the described caffeine-metabolites test.

**Chemicals**

We used an analytical sample of AFMU supplied by Dr. Kalow, Department of Pharmacology, University of Toronto, Toronto, Canada. Ammonium sulfamate, 1-methylxanthine (both from Fluka AG, Buchs, Switzerland), sodium nitrite (Fisher Scientific Co., Pittsburgh, PA), N-1-naphthylenediamine dihydrochloride (J. T. Baker Chemical Co., Phillipsburg, NJ), and hydrochloric acid (BDH Chemicals Ltd., Poole, England) were all as grade. Methanol, acetic acid, and chloroform (all from Fisher Scientific Co., Pittsburgh, PA) were "HPLC" grade. We prepared water for HPLC by passing a "reverse osmosis" water through a trace-organic removal cartridge and 0.45-μm membrane filter (both from Millipore Co., Milford, MA).

**Simplified Caffeine-Metabolites Test**

Sample collection. We collected urine samples from the subjects, all of whom regularly drank coffee, tea, or caffeine-containing cola, about 1 to 6 h after the last ingestion of such liquid. The samples were analyzed immediately or stored frozen at −20 °C until analysis.

Analysis procedure. We analyzed the samples by use of a modified version of the HPLC method described by Grant et al. (13, 14). The chromatograph (Waters Associates) consisted of an autosampler (Model 712 WISP), solvent-delivery pump (Model M-45), a radial-compression module (Model Z) equipped with an 8 × 10 cm C18 NV Radial Pak™ cartridge packed with 4-μm particles and a Guard Pak™ precolumn module with a C18 insert, a variable-wavelength ultraviolet/visible detector (Model 481) set at 280 nm, and a data module (Model 740). We prepared the mobile phase by mixing 880 mL of dilute (50 mL/L) acetic acid and 120 mL of methanol and used a flow rate of 1.2 mL/min. We extracted the metabolites from the 0.2-mL urine sample by adding 6 mL of a mixture of 950 mL of chloroform and 50 mL of isopropanol to a tube containing 120 mg of ammonium sulfate. After vortex-mixing for 10 s, the tube was centrifuged at 3000 rpm for 10 min, and the organic layer was removed and brought to dryness under a gentle stream of nitrogen. We reconstituted the residue with 0.5 mL of the mobile phase, and programmed the autosampler to inject 25 μL into the cartridge. We calculated the peak height ratio of AFMU/1MX directly from the chromatogram and used the value to determine the acetylator phenotype for each patient. The inter- and intra-run precision of the peak height ratio was good (each CV was <4.8%).

**Intrasubject variability of peak height ratio.** To examine the reproducibility of the result obtained with this test, the peak height ratio of AFMU to 1MX was determined for 10 additional subjects by use of urine samples collected from each of these subjects on two different days separated by a period of two to five weeks.

**Sulfasalazine Test**

An oral 500-mg dose of sulfasalazine was given to the subject and a urine sample, collected 6 to 8 h later, was analyzed according to the method described by Schroder (9). The procedure is as follows. Place a 20-μL portion of urine in a glass test tube (Tube I), and another 50-μL portion of the same urine in a similar tube (Tube II). Hydrolyze the urine in Tube I by adding 1 mL of 6 mol/L hydrochloric acid and heating at 80 °C for 5 min. To Tube II add 1 mL of 6 mol/L hydrochloric acid but do not heat. Treat the contents of both tubes with 1 mL of a 1.2 g/L solution of sodium nitrite in water, then after 2 min add 1 mL of an 8 g/L aqueous solution of ammonium sulfamate and 1 mL of an 8 g/L solution of N-1-naphthylenediamine dihydrochloride to each tube. Shake the tubes for 1 min, and visually compare the red-purple color formed in the two tubes.

If the color in Tube I is more intense than that in Tube II, the subject is classified as a fast acetylator; if the color in Tube I is less intense than that in Tube II, the subject is classified as a slow acetylator. For accurate evaluation of the color intensity both tubes should be identical in shape and brand. If the assessment cannot be made visually, the absorbances of the solutions in the tubes are compared by use of a spectrophotometer.

**Results**

Table 1 lists the age and sex of the subjects whose acetylator phenotype was determined by both tests. The mean (and SD) age was 30 (9.1) years and the male/female ratio was 3/1.

Figure 1 depicts a representative chromatogram for an extract of a urine sample collected from one of the subjects. The retention times of AFMU and 1MX were 5.56 and 8.3 min, respectively, and the peaks of both metabolites were well resolved from those of other metabolites or compounds in urine. The peak height ratios of AFMU/1MX (Table 1) exhibited excellent intra-run precision (i.e., CV <2.5%).

Figure 2 shows the peak height ratios obtained on two different days (separated by a period of two to five weeks) for the subjects included in the reproducibility study. The intra-subject variability in the ratio was small, but generally higher CV values are to be expected for low acetylators. For no patient, including the slow acetylator, was the acetylator phenotyping outcome changed.

A frequency distribution histogram of the peak height ratios obtained is shown in Figure 3. The distribution appears to be bimodal with an antimode value of 2.9, which coincides with the first break in the abscissa of the histogram. Thus, the subjects are divided into two groups, 40 (76.9%) being in the slow-acetylator group and 12 (23.1%) in the rapid-acetylator group. These data are in perfect agreement with the acetylator phenotype results obtained with the sulfasalazine test (Table 1) for these subjects. They also agree well with our findings (15) for 297 similar subjects from the Saudi Arabia population.

CLINICAL CHEMISTRY, Vol. 35, No. 5, 1989 849
Table 1. Vital Characteristics of the Subjects, and Comparison between Results Obtained with the Simplified Caffeine Metabolites Test and with the Sulfasalazine Test

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, Sex</th>
<th>PHR&lt;sup&gt;a&lt;/sup&gt; Acetylator Phenotype</th>
<th>Patient No.</th>
<th>Age, Sex</th>
<th>PHR&lt;sup&gt;b&lt;/sup&gt; Acetylator Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>M 0.679 SLOW</td>
<td>27</td>
<td>23</td>
<td>H 2.375 SLOW</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>F 1.081 SLOW</td>
<td>28</td>
<td>31</td>
<td>F 0.328 SLOW</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>H 0.646 SLOW</td>
<td>29</td>
<td>24</td>
<td>H 0.457 SLOW</td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>H 0.778 SLOW</td>
<td>30</td>
<td>40</td>
<td>F 0.760 SLOW</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>F 5.954 FAST</td>
<td>31</td>
<td>30</td>
<td>H 5.393 FAST</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>H 4.481 FAST</td>
<td>32</td>
<td>52</td>
<td>H 7.209 FAST</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>F 4.039 FAST</td>
<td>33</td>
<td>29</td>
<td>H 0.388 SLOW</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>H 4.711 FAST</td>
<td>34</td>
<td>28</td>
<td>H 0.286 SLOW</td>
</tr>
<tr>
<td>9</td>
<td>48</td>
<td>F 0.660 SLOW</td>
<td>35</td>
<td>31</td>
<td>H 2.325 SLOW</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>F 0.496 SLOW</td>
<td>36</td>
<td>33</td>
<td>H 0.752 SLOW</td>
</tr>
<tr>
<td>11</td>
<td>40</td>
<td>H 0.922 SLOW</td>
<td>37</td>
<td>28</td>
<td>H 3.939 FAST</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>H 0.739 SLOW</td>
<td>38</td>
<td>29</td>
<td>H 1.849 SLOW</td>
</tr>
<tr>
<td>13</td>
<td>34</td>
<td>H 0.996 SLOW</td>
<td>39</td>
<td>27</td>
<td>H 0.516 SLOW</td>
</tr>
<tr>
<td>14</td>
<td>19</td>
<td>F 1.261 SLOW</td>
<td>40</td>
<td>28</td>
<td>H 8.312 FAST</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>F 0.994 SLOW</td>
<td>41</td>
<td>21</td>
<td>H 3.752 FAST</td>
</tr>
<tr>
<td>16</td>
<td>28</td>
<td>H 0.651 SLOW</td>
<td>42</td>
<td>27</td>
<td>H 2.662 SLOW</td>
</tr>
<tr>
<td>17</td>
<td>17</td>
<td>F 0.822 SLOW</td>
<td>43</td>
<td>27</td>
<td>H 0.447 SLOW</td>
</tr>
<tr>
<td>18</td>
<td>22</td>
<td>F 2.678 SLOW</td>
<td>44</td>
<td>27</td>
<td>H 0.672 SLOW</td>
</tr>
<tr>
<td>19</td>
<td>26</td>
<td>H 3.909 FAST</td>
<td>45</td>
<td>21</td>
<td>H 0.625 SLOW</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>H 1.027 SLOW</td>
<td>46</td>
<td>28</td>
<td>H 0.706 SLOW</td>
</tr>
<tr>
<td>21</td>
<td>28</td>
<td>H 3.732 FAST</td>
<td>47</td>
<td>27</td>
<td>H 0.861 SLOW</td>
</tr>
<tr>
<td>22</td>
<td>21</td>
<td>F 2.857 SLOW</td>
<td>48</td>
<td>27</td>
<td>H 2.352 SLOW</td>
</tr>
<tr>
<td>23</td>
<td>22</td>
<td>H 0.481 SLOW</td>
<td>49</td>
<td>26</td>
<td>H 0.687 SLOW</td>
</tr>
<tr>
<td>24</td>
<td>26</td>
<td>H 0.942 SLOW</td>
<td>50</td>
<td>23</td>
<td>H 0.607 SLOW</td>
</tr>
<tr>
<td>25</td>
<td>33</td>
<td>H 5.751 FAST</td>
<td>51</td>
<td>30</td>
<td>H 0.570 SLOW</td>
</tr>
<tr>
<td>26</td>
<td>32</td>
<td>F 1.724 SLOW</td>
<td>52</td>
<td>23</td>
<td>H 0.321 SLOW</td>
</tr>
</tbody>
</table>

<sup>a</sup>Peak height ratio of AFMU/1MX; for slow acetylator PHR < 2.9 and for rapid acetylator PHR > 2.9.

<sup>b</sup>As determined by the sulfasalazine test.

---

Fig. 1. A representative chromatogram of a standard solution (S) of AFMU (I) and 1MX (II) and an extract (P) of a urine sample from a rapid-acetylator subject.

Fig. 2. Intra-subject reproducibility of the peak height ratios.

Fig. 3. A frequency distribution histogram of the peak height ratio AFMU/1MX for subjects whose acetylator phenotype was also determined by sulfasalazine test.
Discussion

Because the sulfasalazine procedure used in this study requires no special equipment for determination of acetylator phenotype, it has been described by Schroder (9) as "simplified." However, this method, like other acetylator phenotyping tests, requires the administration of a sulfonamide and collection of a urine or plasma sample a certain interval after ingestion of the drug. This has proved to be unpopular for large-scale population studies. In contrast, because the AFMU/1MX ratio approaches no maximum value and the concentrations of these metabolites are relatively insensitive to variation in oral absorption or renal excretion, the caffeine metabolite test apparently does not require careful monitoring of the amount of caffeine intake or timing of the urine collection (14). For these reasons, and because caffeinated beverages are considered food items and not drugs, subjects ordinarily do not refuse or hesitate to participate in such studies.

Owing to the excellent linearity of the relation between the above parameters for AFMU/1MX, we have shown in this report that this peak height ratio can be used to determine the acetylator phenotype in lieu of the molar concentration ratio, obviating the time and effort needed to prepare the standard curve and calculate this ratio. Indeed, by use of this approach one can accurately and reliably (Table 1) determine the acetylator phenotype in less than 30 min, which is the shortest time reported for such a test, thus making it highly suitable for population studies.

We thank the administration of the King Faisal Specialist Hospital & Research Center for its support of the pharmacokinetics research program at this institution.

References


CLIN. CHEM. 35/5, 851-856 (1989)

Concentrations of Some Trace Elements (Se, Zn, Cu, Fe, Mg, K) in Blood and Heart Tissue of Patients with Coronary Heart Disease

O. Oester,1 M. Daehm,2 H. Oelert,2 and W. Prehlwitz1

We measured Se, Zn, Fe, Cu, Mg, and K in blood and heart tissue of patients with coronary heart disease. Such patients have subnormal selenium concentrations in serum, whole blood, and (calculated per gram of hemoglobin) erythrocytes. Concentrations of zinc and copper in serum were also subnormal in these patients. Heart tissue collected from these patients during bypass surgery was analyzed for Se, Zn, Fe, Cu, Mg, and K; results are expressed in terms of wet weight and in relation to nitrogen and phosphorous content. Concentrations of these elements in blood are correlated with those in heart tissue. Selenium concentrations in serum correlated positively with those in tissue but not with those in erythrocytes. We found no association between concentrations of zinc, iron, copper, magnesium, and potassium in serum and the corresponding concentrations in heart tissue. There was a moderately positive correlation between the concentration of ferritin in serum and that of iron in tissue. We conclude that the turnover rate for selenium in tissue is similar to that in serum but greater than that for erythrocyte selenium. The concentrations of these six elements in heart tissue are partly correlated with the ejection fraction of the left ventricle.

Additional Keyphrases: ejection fraction • tissue analysis • atomic absorption spectrometry • ferritin

Selenium, an essential trace element, is part of the enzyme glutathione peroxidase (EC 1.11.1.9), which is involved in removal of hydrogen peroxide and lipid peroxides produced during oxidative processes in cells. Glutathione

CLINICAL CHEMISTRY, Vol. 35, No. 5, 1989 851