A shortened, 2-hour rifampin test: a useful tool in Gilbert's syndrome

H. Hallal\textsuperscript{a}, J.M. Egea\textsuperscript{b}, P. Mas\textsuperscript{a}, M.D. García\textsuperscript{b}, E. Pérez-Cuadrado\textsuperscript{a} and F. Carballo\textsuperscript{a}

\textsuperscript{a}Digestive Diseases Section. Hospital General Universitario Morales Meseguer. Murcia. Spain.
\textsuperscript{b}Clinical Chemistry Service. Hospital General Universitario Morales Meseguer. Murcia. Spain.

SUMMARY

INTRODUCTION: Diagnosis of Gilbert’s disease often involves unnecessary testing and patient anxiety. Rifampin test can support the diagnosis; it has been described in short series and lacks standardization in dose, collection times, result presentation and interpretation. Our objective was to compare the response to oral rifampin in a series of patients with Gilbert’s disease, 2 and 4 h after drug administration.

PATIENTS AND METHODS: Eighty-nine patients with Gilbert’s disease (elevated total bilirubin with no hepatopathy or hemolysis) were recruited. After a basal blood collection, 900 mg rifampin were administered \textit{per os} and new samples were drawn 2 and 4 h later. Total and esterified bilirubin were measured in every sample. Haptoglobin concentration was also analyzed.

RESULTS: When expressed as relative increase with respect to basal values, variations observed 2 h after rifampin intake were all above 15%. A significant correlation ($r = 0.902$; $p = 0.000$) was found between relative increases 2 and 4 h after drug administration. No significant variations were found in haptoglobin concentrations.

CONCLUSION: Rifampin test is useful in diagnosing Gilbert’s disease, but variations in total bilirubin concentrations (basal and post-rifampin) make that no absolute cut-off value can be used. Correlation between 2- and 4-h relative increases suggests that a shortened version could simplify the test.

INTRODUCCIÓN. El diagnóstico de la enfermedad de Gilbert conlleva, a menudo, la realización de pruebas innecesarias que incrementan la ansiedad del paciente. La prueba de la rifampicina puede apoyar el diagnóstico. Esta prueba ha sido descrita en grupos pequeños de pacientes y todavía carece de estándarización en lo relativo a los dosis, los momentos de obtención de las muestras de sangre, la presentación del resultado y su interpretación.

PACIENTES Y MÉTODOS. En el estudio participaron 89 pacientes con enfermedad de Gilbert (incremento de la concentración de bilirrubina total sin hepatopatía ni hemólisis). Tras la obtención de una muestra inicial de sangre, se administraron 900 mg de rifampicina por vía oral y, posteriormente, a las 2 y a las 4 horas de esta administración, se volvieron a obtener muestras de sangre. En cada muestra se determinaron las concentraciones de bilirrubina total y de bilirrubina esterificada. También se determinó la concentración de haptoglobina.

RESULTADOS. Mediante su expresión como el incremento relativo respecto a los valores basales, todas las variaciones observadas a las 2 horas de la administración de rifampicina fueron superiores al 15%. Se observó una correlación significativa ($r = 0.902$; $p = 0.000$) entre los incrementos relativos detectados a las 2 y a las 4 horas de la administración del medicamento. No se detectaron variaciones significativas en las concentraciones de haptoglobina.

CONCLUSIÓN. La prueba de la rifampicina es útil para establecer el diagnóstico de la enfermedad de Gilbert, pero las variaciones en las concentraciones de bilirrubina total (basal y tras la administración de rifampicina) no permiten establecer un valor umbral absoluto. La correlación observada entre los incrementos relativos a las 2 y a las 4 horas indica que la versión breve de la prueba (2 horas) podría simplificar su aplicación.

INTRODUCTION

Gilbert’s syndrome is a frequent hereditary chronic and benign disorder characterized by unconjugated hyperbilirubinemia in the absence of structural liver disease or
because hemolysis exerts a strong negative analytical interference on the pro-

Characteristics of the 78 included patients

(25 women, and 53 men)

shortened, 2-h version of the test shows an equally useful

Therefore, we studied the progression of total bilirubin at

for the rifampin test to be useful in Gilbert's syndrome.

solute increase in total bilirubin concentration are needed

individual biological variation. Moreover, available data

µmol/l (1.9 mg/dl). However, many patients start from

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline

Unconjugated bilirubin (µmol/l) 22.23 ± 7.35 10.26-46.17

Unconjugated bilirubin (µmol/l) 22.23 ± 7.35 10.26-46.17

overt hemolysis. Diagnosis is made by exclusion, and no
diagnostic test has been adopted as a universally accepted
reference. However, the need for a follow-up confirma-
tion of the absence of disease explains the use of a variety
of tests that support the initial diagnosis; among them,
there are tests based on the bilirubin response to fasting,
nicotinic acid, phenobarbital or rifampin. The last one is

Mathematical and statistical methods

Concentration of unconjugated bilirubin was calculated as the difference
between the measured concentrations for total and conjugated bilirubin.
In order to standardize results, and to avoid the effects of intra- and in-
travidual biological variation, response to rifampin was expressed as relative
increases in total bilirubin concentration at 2 and 4 h, with respect to basal
delay. Relationship between relative increases at 2 and 4 h was analyzed by
Spearman's correlation.

RESULTS

After administration of 900 mg rifampin, concentrations of total and unconjugated
bilirubin expressed as mean and 95% confidence interval (95% CI) were, respecti-
vely, 43.8 (95% CI 22.4-61.7) and 29.4 (95% CI 14.5-
51.0) µmol/l at 2 h, and 55.1 (95% CI 32.8-80.4) and 37.8
(95% CI 18.8-64.8) µmol/l at 4 h. Thirty-one subjects
had a basal concentration of total bilirubin above 32.5
µmol/l (1.9 mg/dl). No other patient sample. Variations due to analytical bias or random error
were within the acceptable criteria according to external quality assur-
ance programs.

HALLAH H. ET AL. A SHORTENED, 2-HOUR RIFAMPIN TEST: A USEFUL TOOL IN GILBERT'S SYNDROME

Fig. 1. Relative increase in total bilirubin at 2 and 4 h after rifampin
administration (900 mg). BT0: basal total bilirubin; BT2: total bilirubin
after 2 h; BT4: total bilirubin after 4 h.

TABLE I. Characteristics of the 78 included patients (25 women, and 53 men)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.74 ± 15.46</td>
<td>15-80</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>19.83 ± 7.99</td>
<td>10-44</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>20.93 ± 4.66</td>
<td>15-34</td>
</tr>
<tr>
<td>ALP (IU/l)</td>
<td>176.01 ± 74.13</td>
<td>83-600</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>15.60 ± 8.30</td>
<td>6-42</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>263.46 ± 57.37</td>
<td>126-462</td>
</tr>
<tr>
<td>Total bilirubin (µmol/l)</td>
<td>30.78 ± 9.23</td>
<td>13.66-53.53</td>
</tr>
<tr>
<td>Unconjugated bilirubin (µmol/l)</td>
<td>22.23 ± 7.35</td>
<td>10.26-46.17</td>
</tr>
</tbody>
</table>

Methods

Procedure for rifampin test

After a 12-h fasting, a basal blood sample was collected, and immedi-
ately 900 mg rifampin were administered per os. New blood collections
were made at 2 and 4 h after rifampin intake. A catheter was inserted to
avoid repeated punctures. Patients were not allowed to eat or drink du-
ting the test.

Analytical procedures

Total and conjugated bilirubin were measured in all samples. In addi-
tion, haptoglobin was measured in basal and 4-h samples in order to in-
vestigate a possible hemolytic effect of rifampin during the test. Measu-
rements were carried out on an Advia® 1650 analyzer (Bayer Diagnostics,
Tarrytown, New York, USA), according to the procedures, reagents and calibrators provided by the manufacturer. Methods for bili-
rubin (total and conjugated) were those described by Jendrassik and
Goetz3 as for haptoglobin, the method was immunoturbidimetrica.

Samples were analyzed within 2 h from their collection, included in the

Laboratory routine and not as a separate series; thus, manipulation, re-
cord, centrifugation and analytical processing were the same as any
other patient sample. Variations due to analytical bias or random error
were within the acceptable criteria according to external quality assur-
ance programs.

Patients and methods

PATIENTS AND METHODS

Patients

On a prospective basis, we studied 89 patients admitted to the specia-
list’s office in the digestive disease service of a general hospital with
clinical diagnosis of Gilbert’s syndrome. Criteria for inclusion were: to-
tal bilirubin concentration greater than 22.2 µmol/l (1.3 mg/dl), with a
conjugated bilirubin concentration normal or slightly elevated (less than
twice in the last 6 months); absence of analytical data compatible with hepa-
tic or hemolytic disease (aminotransferases, gamma-glutamyl trans-
ferase, alkaline phosphatase, lactate dehydrogenase, haptoglobin, blood
cell count, reticulocytes, prothrombin time); no excessive alcohol con-
sumption (less than 20 g/day) or hepatotoxic drug intake; and normal li-
ter ultrasonography.

Pregnancy, lactation or allergy to rifampin were established as criteria
for exclusion; 11 patients were excluded a posteriori because no overt
hemolysis rendered possible some of the collected samples useless, since in

overt hemolysis exists a strong negative analytical interference on the pro-
cedure used for total bilirubin measurement. Characteristics of the sub-
ject group are shown in table I.
patients presented at least an increase of 15% at 2 h and 38% at 4 h. A positive correlation was found between the relative increases at 2 and 4 h (Spearman’s rho = 0.902; p < 0.000).

DISCUSSION
Gilbert’s syndrome is probably inherited as an autosomal dominant trait and affects 5-7% of total population, more often in males. Diagnosis of Gilbert’s syndrome is based on exclusion rather than on a panel of tests1,2. Many different tests exist that can support the diagnosis, like those based on fasting, phenobarbital, nicotinic acid or rifampin, and controls, although in a later communication they stated that rifampin is useless in diagnosing Gilbert’s syndrome as compared to fasting test. Velilla et al3,11 drew blood at a basal state and 4 h samples after rifampin intake. Hence, we suggest that the relative increase of total bilirubin at 2 h can be useful in the diagnosis of Gilbert’s syndrome.

In conclusion, according to our results: a) all patients with Gilbert’s syndrome showed a relative increase in total bilirubin concentration of at least 0.15 times their basal values, 2 h after rifampin administration; b) relative increases at 2 and 4 h are correlated and thus can give similar information, and c) there were no change in haptoglobin concentration between samples collected at basal state and 4 h samples after rifampin intake. Hence, we believe that the number of patients with Gilbert’s syndrome give a relative increase in total bilirubin concentration of at least 0.15 times their basal values, 2 h after rifampin administration; relative increases at 2 and 4 h are correlated and thus can give similar information, and c) there were no change in haptoglobin concentration between samples collected at basal state and 4 h samples after rifampin intake. Hence, we suggest that the relative increase of total bilirubin at 2 h can be useful in the diagnosis of Gilbert’s syndrome.

REFERENCES