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

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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 *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.

PRUEBA BREVE (2 HORAS) CON RIFAMPICINA: UNA HERRAMIENTA ÚTIL EN EL SÍNDROME DE GILBERT

OBJETIVO. Comparar la respuesta a las 2 y a las 4 horas de la administración oral de rifampicina en una serie de pacientes con enfermedad de Gilbert.

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 estandarización en lo relativo a la 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

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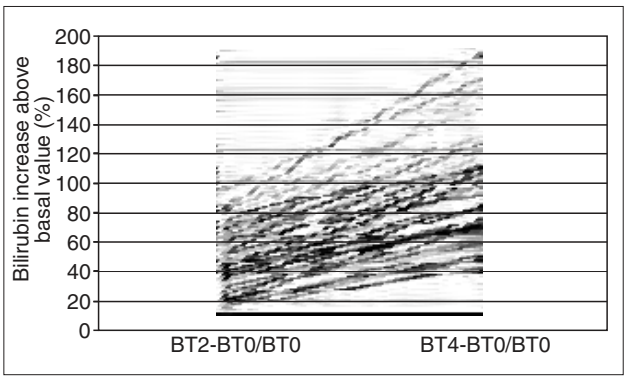

Fig. 1. Relative increase in total bilirubin at 2 and 4 h after rifampin administration (900 mg). BT0: basal total bilirubin; BT2: total bilirubin after 2 h; BT4: total bilirubin after 4 h..

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

	Average ± SD	Range
Age (years)	34.74 ± 15.46	15-80
ALT (IU/l)	19.83 ± 7.99	10-44
AST (IU/l)	20.93 ± 4.66	13-34
ALP (IU/l)	176.01 ± 74.13	83-600
GGT (IU/l)	15.60 ± 8.30	6-42
LDH (IU/l)	293.46 ± 57.37	126-462
Total bilirubin (μmol/l)	30.78 ± 9.23	13.68-53.35
Unconjugated bilirubin (μmol/l)	22.23 ± 7.35	10.26-46.17

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; SD: standard deviation.

overt hemolysis[1]. Diagnosis is made by exclusion, and no diagnostic test has been adopted as a universally accepted reference. However, the need for a follow-up confirmation 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 the most frequently performed in our setting. Pérez et al[2] reported that nicotinic acid and rifampin tests are comparable in the diagnosis of Gilbert's syndrome; it is nevertheless important to point out that no consensus exists about the test procedure and the interpretation of results.

Rifampin test normally takes 4 h and is considered as positive if total bilirubin concentration rises above 32.5 μmol/l (1.9 mg/dl). However, many patients start from even higher basal levels, due to a marked inter- and intra-individual biological variation. Moreover, available data show that the major relative increase in total bilirubin concentration occurs in the first 2 h, and is less intense afterwards (4 to 6 h)[3]. Thus, magnitudes other than the absolute increase in total bilirubin concentration are needed for the rifampin test to be useful in Gilbert's syndrome. Therefore, we studied the progression of total bilirubin at 2 and 4 h after rifampin administration, in a sufficiently large series of patients with a clinical diagnosis of Gilbert's syndrome, with the aim to estimate whether the shortened, 2-h version of the test shows an equally useful pattern.

PATIENTS AND METHODS

Patients

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

Pregnancy, lactation or allergy to rifampin were established as criteria for exclusion; 11 patients were excluded *a posteriori* because *in vitro* hemolysis rendered some of the collected samples useless, since *in vitro* hemolysis exerts a strong negative analytical interference on the procedure used for total bilirubin measurement. Characteristics of the subject group are shown in table I.

Methods

Procedure for rifampin test

After a 12-h fasting, a basal blood sample was collected, and immediately 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 during the test.

Analytical procedures

Total and conjugated bilirubin were measured in all samples. In addition, haptoglobin was measured in basal and 4-h samples in order to investigate a possible hemolytic effect of rifampin during the test. Measurements were carried out in an Advia®-1650 analyzer (Bayer Diagnostics, Tarrytown, New York, USA), according to the procedures, reagents and calibrators provided by the manufacturer. Methods for bilirubin (total and conjugated) were those described by Jendrassik and Grof[4]; as for haptoglobin, the method was immunoturbidimetric[5].

Samples were analyzed within 2 h from their collection, included in the laboratory routine and not as a separate series; thus, manipulation, recording, 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 assurance programs.

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 inter-individual biological variation, response to rifampin was expressed as relative increases in total bilirubin concentration at 2 and 4 h with respect to basal values. 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, respectively, 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 (95% CI, 1.9 mg/dl). On calculating relative increases (related to the basal value) 2 and 4 h after drug intake, it can be observed, as shown in figure 1, that all pa-

tients 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[6]. Diagnosis of Gilbert's syndrome is based on exclusion rather than on a panel of tests[7]. Many different tests exist that can support the diagnosis, like those based on fasting, phenobarbital, nicotinic acid or rifampin. These 2 latter are comparable[2]. The last one has been carried out using varied doses and collection timing. Murthy et al[3] gave 900 mg rifampin fasting, with blood drawing at 2, 4 and 6 h, and considering a total bilirubin concentration greater than 32.5 µmol/l (1.9 mg/dl) to discern between subjects without and with Gilbert's syndrome. In contrast, Erdil et al[8] administered 600 mg rifampin and made bilirubin measurement 4 h later, assuming as a positive response a 50% increase in unconjugated bilirubin above its basal value. Noguerado et al[9] utilized a 300 mg dose and collected samples 3 h post-rifampin, but they set no threshold value and concluded that rifampin is useless in diagnosing Gilbert's syndrome as compared to fasting test. Velilla et al[10] drew blood at a basal point and 1, 2, 3 and 4 h after a 900 mg rifampin intake and did not establish a cut-off point between patients and controls, although in a later communication they stated that a decision level had been set at 29.1 µmol/l (1.7 mg/dl)[7]. Pérez et al[2] used 900 mg rifampin and made basal and hour-by-hour collections until 4 h choosing no threshold value. Atmetlla et al[11] administered 900 mg rifampin and selected neither cut-off limit.

In our study, all patients with Gilbert's syndrome showed a relative increase in total bilirubin concentration of at least 0.15 times their basal values at 2 h, and 0.38 at 4 h. As for haptoglobin levels, these were measured in 72 patients and the mean basal value was 0.98 g/l (95% CI, 0.10-2.83) and 4 h after rifampin administration 0,97 g/l (95% CI, 0.07-2.83) /L showing no difference, unlike the results obtained by Murthy et al[3].

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 suggest that the relative increase of total bilirubin at 2 h can be useful in the diagnosis of Gilbert's syndrome.

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