ISONIAZID BIOTRANSFORMATION IN A POPULATION OF PATIENTS WITH PULMONARY TUBERCULOSIS

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Summary

Many *Mycobacterium tuberculosis* infections are currently observed. Several of these infections are resistant to antifimic drugs such as Isoniazid (INH) and an insufficient dosing of the compound due to a lack of adherence to treatment can cause low levels of the drug especially in rapid acetylators. The number of rapid and slow acetylators in a population starting treatment for pulmonary tuberculosis was measured once they received the first dose of INH. A sample of urine was collected from these persons and the ratio acetylisoniazid/free Isoniazid (AcINH/INH) was measured. Sixty seven patients were rapid acetylators and 44 patients were slow acetylators. The group of rapid acetylators had an average AcINH/INH ratio value of 85 ± 9% and slow metabolizers had an average activity of 48 ± 13%. Both types of acetylators were present in important relative proportions in this population. It is thought that, when these differences are not taken into account, the acquired resistance to INH in mycobacterium can establish sooner in rapid acetylators and a more probable presence of toxicity can appear in slow metabolizers. In both cases, preventive measures should be provided.

Key Words: Isoniazid, acetylating rate, pulmonary tuberculosis

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Introduction

Pulmonary tuberculosis (PTB) is an infectious disease produced mainly by *Mycobacterium tuberculosis* and is a severe problem of Public Health. In Mexico, in 2005, 15,249 cases of pulmonary tuberculosis were registered, 97% of them were young people at a productive age (1). A series of factors have determined that the infection has remained without being eradicated and at the present time it is considered as a very real concern in many countries, both, developed and those on their way to develop. These factors can be identified as much in the pathogenic microorganism, deficient mechanisms of defense, little adhesion to treatment, and possibly, low plasmatic concentrations due to the kinetic characteristics of some persons (2).

Isoniazid (INH), singly (in prophylaxis) or in combination with other drugs continues being the antifimic drug most used in the first line treatment of PTB. The drug is eliminated in urine mainly as the acetylated metabolite (AcINH). INH is metabolized in non-homogeneous form to AcINH by the fast or slow activity of the polymorphic enzyme *N*-acetyltransferase 2 (NAT2) (3).

The most recommended form of administration is as a directly observed ingestion of medication under the supervision of health personnel (directly observed treatment; DOT). This scheme of treatment was proposed (4) to improve the outcome in PTB treatment either on a daily basis or when bi- or tri-weekly modes were prescribed; and when strictly followed, it proposes effective concentrations of antituberculosis drugs to obtain a cure. When not followed strictly, the possibility exists of a resistance to the drug caused by low plasma concentrations resulting from an accelerated metabolism in fast acetylators or the possibility of appearance of toxic effects by increased plasma concentrations of INH in drug slow acetylators.

On the other hand, several reports (5, 6) indicate the possibility of toxic effects of isoniazid (INH) when larger doses of the drug are ingested either for suicidal purposes or by over ingestion to cover missed doses. These amounts of INH could cause toxic effects in slow acetylators.

Acetylation rate has marked variations in different races; individuals of Asian origin including Japanese and Eskimos are mainly fast acetylators (7). These fast metabolizers are people who in only a few hours biotransform this compound to produce non antituberculous molecules. Some racial groups are mainly slow acetylators; for instance it has been informed that 95% of a Saudi population had the slow acetylator phenotype (8). Recent reports on race-based variability of the acetylator status in Caucasian populations found that 50% to 60% of them were slow acetylators (9). In a study reported by Gross et al. (10) from a Caucasian-American population, 58.1% and 59.5% of the subjects were classified phenotypically and genotypically, respectively, as slow acetylators. In a study made in a population of Nigeria, it was observed that 61.9% of the subjects were fast acetylators (11). In another study made on a Spanish population, 65.6% were slow acetylators and 34.3% were fast acetylators (12). There are certain studies done with Mexican populations mentioning their acetylator status evaluated in the process of determining antinuclear antibodies in relation to a lupus syndrome induced by drugs like INH in a restricted Mexican population with tuberculosis (13, 14) who reported a fast acetylator phenotype in approximately 50% of their sample population and a study with sulfamethazine (15) showed, in healthy volunteers, a considerable percentage of both, rapid and slow acetylators.

A toxicity due to a high dose of INH could appear in those people who slowly biotransform this compound and the possibility exists that interactions with other drugs by a larger permanence of the compound in the organism can occur (16). In the case of fast acetylators, the INH has a shorter half-life in plasma and therefore a concentration would be achieved smaller than the reported minimal inhibitory concentration (MIC) of 0.025 to 0.05 µg/ml (17).

The purpose of this study was to know the proportion of rapid and slow acetylators in a selected population and to consider the implications of non-adherence to the recommended treatment schemes for PTB if a substantial number of rapid or slow acetylators are present in any given population which receives treatment with INH.

Methods

This was a study, in which, urine samples were collected from patients suffering PTB and their close contacts with a range of 20 to 75 years of age, attending their family clinic in Mexico, that upon diagnose, received INH for the first time as a single drug. The patients were informed on the objectives of the study and accepted to participate in it as required by the Ethics Committee of our Institution. Each patient in a fasted state, was orally administered a single dose of 300 mg of INH in the form of tablets (Isoniazid Tablets, USP; VersaPharm; Eatontown, NJ, USA) and a urine sample was collected six hours after the ingestion of the compound. Crystals of thymol were added to the tubes as a preservative and samples were stored at -20°C until analyses were done. After the samples were taken, close contacts continued prophylactic treatment with INH, tuberculosis patients on the other hand, immediately continued on standard treatment with two or more drugs.

Exclusion criteria

Samples from patients who were eliminating glucose in urine were discarded since this molecule interferes with the estimation of AcINH. Glucose in urine was determined by means of Combur[10] Test (Roche Diagnostic, Mannheim, Germany) reactive strips. Ingestion of other drugs, excluded also the analyses for those patients.

Estimation of AcINH/INH ratio in urine

An analysis was done on a urine sample after six hours from the ingestion of the first single dose of INH by the method of Eidus et al. (18) based on the estimation of the relative amounts of AcINH and free INH excreted in urine (AcINH/INH).

Transformation of the data and statistical analysis

All values were expressed as the mean ± the corresponding standard deviation. The data obtained in percentage were arcsine transformed and analysis of variance was performed using the SPSS 11.5 for Windows software (SPSS Inc; Chicago, IL. USA) to observe any possible differences between variables. The value for the acceptance of a valid difference was $p < 0.05$.

Results

One hundred and thirty six urine samples were received: 66 men and 70 women. The mean age was 37 years ± 15.3 (range of 21 to 74 years). From that number of patients, 23 were excluded from the study since they presented glucose levels of several magnitudes in urine (16 men and 7 women). Two patients referred a previous ingestion of Rifampin and were also excluded from the study.

Of the 111 analyzed samples, 49 were men and 59.2% of them were classified (according to the proposed method) as fast acetylators. Of the 62 women, 61.3% were classified as fast acetylators.

The mean percent activity estimated by the AcINH/INH ratio in fast acetylator men was of 86.8 ± 8.5% and in women of 84 ± 9.4%, without any significant difference between them ($p > 0.05$).

Slow acetylator men had a mean activity value of 45.6 ± 14% and in the women it was of 50.2 ± 11%, not showing any significant difference between them ($p > 0.05$).

From the total of patients, 67 (60.4%) were fast acetylators (29 men and 38 women); whereas 44 patients (39.6%) were slow acetylators (20 men and 24 women). The group of fast acetylators presented 85 ± 9% of average activity and the slow ones had an average of 48 ± 13% activity. In Figure 1 it can be seen the frequency distribution of fast and slow INH acetylators.

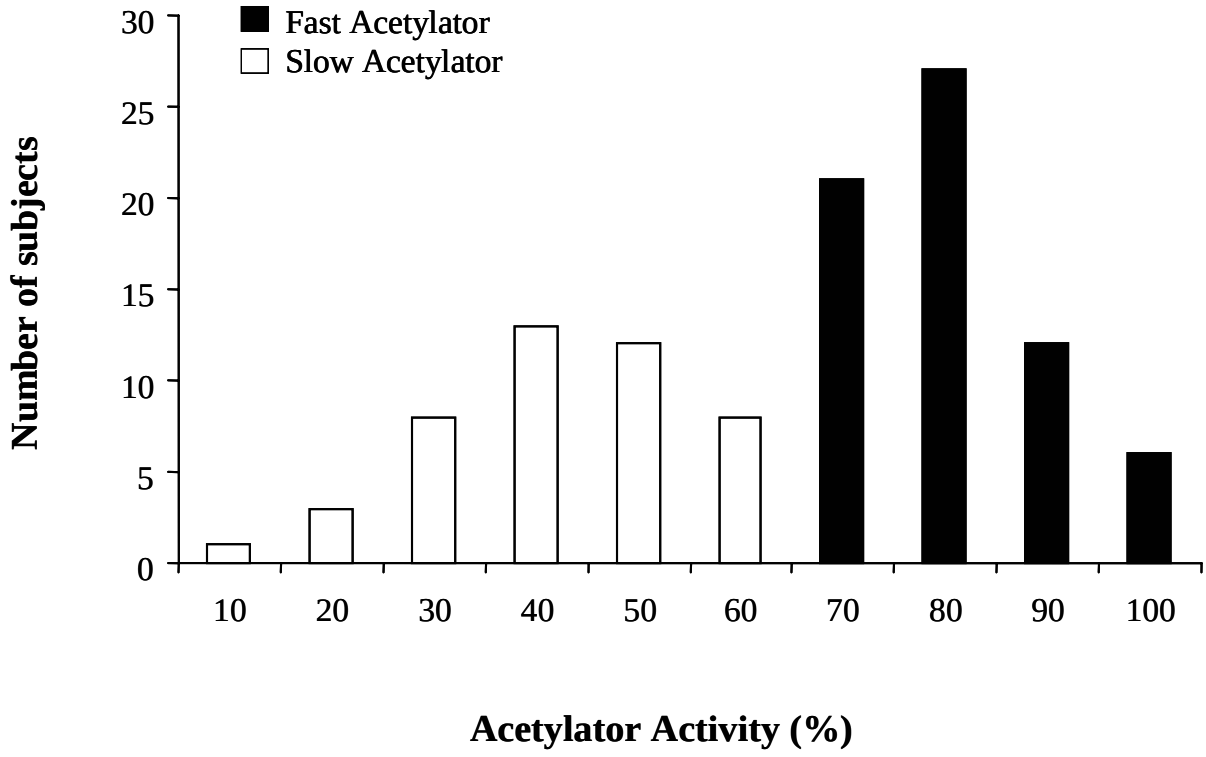

Figure 1: Frequency histogram of all individuals according to their acetylator activity. Slow and Fast acetylators are indicated.

Discussion

Pulmonary tuberculosis is an infectious disease that has not been well controlled in many countries and that has probably resurged by virtue of several factors; among them, an observed higher incidence of infections due to mycobacterium bacilli resistant to one or several of the antituberculous drugs (4).

Our study was done in a human population in Mexico with essentially similar socio-economic status, who started treatment with INH both, as a single drug (prophylaxis) or as a part of a multidrug treatment. The precise birth origin of these persons was not considered since we were interested only in the relative numbers of acetylation rates in persons from that population and not in the implications of their genetic composition. In this study we used the method of Eidus et al. (18) which is a useful, not expensive, non invasive procedure that uses a urine sample of patients that take INH.

The results of the study showed that 60.4% from the total population were fast acetylators and 39.6% were slow acetylators. These results are only slightly different from those found with other objectives by Alarcon-Segovia et al. (13) in Mexican patients with PTB who reported a fast acetylator phenotype in approximately 50% of their sample population.

Several studies have shown great variation in the acetylator phenotype in different ethnic groups. As mentioned in the Introduction, there are publications on the race-based variability of the acetylator status in many populations. We were interested in knowing the number of fast and slow acetylators in a population in order to propose modifications in the frequency of administration of INH especially when this drug is used as a monotherapy.

In the present study we observed that in the group considered as rapid acetylators (60.4% of the sampled population), more than 80% of the INH was metabolized before the 6 hours period (86.8% in men and 84% in women). In the slow acetylators group the INH was biotransformed to an extent of 45.6% in men and 50.2% in women, in the same period. The plasma concentrations of INH in slow acetylators taking a dose of 5 mg/kg with a peak plasma concentration of 7.1 µg/ml one hour after the ingestion of INH would be of 0.028 µg/ml, after almost 24 h, given their half life of 3.1 h (19), above the lower limit of the reported MIC (17). Conversely, fast acetylators of INH with a 1 h peak plasma concentration of 5.4 µg/ml, at 24 h would have plasma concentrations (2.6 pg/ml), much lower than the MIC given their half-life of 1.1 h (19).

Accordingly, it becomes very useful for the physician in charge of any patient to know the phenotype any person has in order to prescribe the most appropriate dosing and to obtain the maximal effectiveness in PTB without the risk of producing undesirable effects in the patient, assuming a strict compliance of the so-called DOT which was designed to assure optimal concentrations of antituberculosis drugs in patients.

As we stated above, when these guidelines are not followed for whatever the reason, the risk exists that in those patients with rapid rates of acetylation, the optimal bactericidal concentration is not obtained and consequently the appearance of resistance is more probable. On the other hand, those patients characterized as slow acetylators can be at the risk of presenting unwanted toxicological collateral damage caused by high plasmatic levels of INH due to several reasons such as suicidal, over ingestion, etc. (20, 21).

Some authors think that the fast acetylators rapidly form toxic metabolites but these are also rapidly turned into non-toxic compounds or they are quickly eliminated. Slow acetylators present an accumulation of these toxic metabolites probably responsible for most of INH toxicity, and it has been observed that these persons tend to suffer peripheral neuropathies more frequently (20).

In agreement with the objective for this study, the knowledge we obtained could be applied in the treatment of PTB. It is possible to optimize the INH treatment by personalizing the doses of the antituberculosis drug according to the phenotypic characteristics of acetylation that any patient has.

In view of our results showing an almost equal distribution of acetylating type of activity regardless of gender, a possible action should be to know the acetylator status of the population. Probably, an explanation of increased resistance of *M. tuberculosis* to INH could be the lack of adherence to treatment, especially in fast acetylators; therefore, it should reinforce the need to a very strict observation of the patient compliance to treatment.

The presence of slow acetylators would make physicians in charge to personalize the scheme of treatment in such patients, making use of more frequent laboratory examinations, such as ALT, AST, etc, and prescribing pyridoxine to prevent additional damage since it is known that this compound antagonizes the toxic effects of INH (6).

Current schemes for the treatment of tuberculosis include the need to be certain of the compliance to treatment by the patient in order to optimize the scheduled dose and to make shorter the exposure to potentially toxic drugs (4). Personalization of treatment in the above terms could be useful to prescribe the best for patients that suffer tuberculosis.

When this knowledge is extrapolated to larger populations, it would serve in some cases to better adjust the dosing of the drug by individualizing it in order to achieve therapeutic concentrations of INH at the *M. tuberculosis* environment in the infected tissue.

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