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ACETYLATION PERCENTAGE METHOD FOR DETERMINATION OF ACETYLATOR STATUS IN HUMAN VOLUNTEERS AND TUBERCULOUS PATIENTS

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ABSTRACT

Acetylation percentage method described here is rapid and economical. A comparison has been made between plasma half life (t 1h), acetylation ratio and acetylation percentage methods for the determination of phenotyping. Isoniazid (INH) was selected as a test drug. In 120 healthy volunteers and 60 tuberculosis patients, acetylation percentage method has been found simple and reliable as compared to the other methods.

Introduction

Acetylation is a phase H drug metabolic reaction occurring in the liver. The disposition and toxicity of many hydrazine, aromatic amino drugs and xenobiotics that make their *way* into the human body are influenced by the rate and extent of N-acetylation (Evans et a1., 1960).

The ability of drugs such as, isoniazid (INH), procainamide, dapson, sulphamethazine, sulphapyridine, phenelzine, etc., to N-acetylate is genetically deter-mined and the trait is inherited in an autosomal dominant gene (Kalow, 1962).

The quantitative assessment of the rate and extent of acetylation of such *drugs* yield bimodal distribution, representing fast and slow acetylators (Evans, 1969 and Gelber et a1., 1977).

The clinical implications of acetylator status are becoming increasing important as slow acetylators have been reported to be at a greater risk of developing side effects, such as drug-induced systemic lupus erythematosus and or isoniazid induced polyneuritis (Food et al., 1977; Fishbein and Segovia, 1979; Woosely et al., 1978; Boobies, 1979).

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The fast acetylators produce higher levels of N-acetylated metabolines. A few among these metabolites are biologically active or act as reactive inter-mediates in pathways with toxic and products (Mitchell et al., 1975).

A variety of substrates have been used to determine the acetylator status (Hutchings and Poutledge, 1986; Hauson et al., 1981; Ellard and Gammion, 1973; Ran et a1, 1970; Ellard et al., 1972). Method like plasma isoniazid half life and plasma acetyl-INH/INH ratio have been used in the past for the determination of acetylator status (Hutchings and Poutledge, 1986), which are time consuming. In this study, a simple and quicker method is reported for the determination of acetylator status using acetylation percentage of isoniazid in 0-3 hours urine.

Experimental

Chemicals:

All chemicals used in this study were of analytical grade, purchased from market.

Isoniazid was donated by Wellshire Laboratories Pakistan Ltd, acetylisoniazid was synthesized using a method reported elsewhere (Mitchell 1975).

Selection of Subjects:

The subjects in the proposed study were divided into two groups, A and B. Group A consisted of 120 healthy young volunteers (45 females and 85 males) in age group 20 to 30 years with 55 to 60 kg body weights. While Group B consisted o tuberculosis patients (20 females and 40 males) in the age group 25 to 70 years and to 70 kg body weights, admitted in the Chest Diseases Unit at Nishter Medical Coll teaching Hospital, Multan. All the subjects involved in this study were informed about the purpose of the study and written consents *were* obtained.

Administration of drug:

Group A

The subjects in this group were restricted from all other medications 7 before, and during the study period. Following an over night fast, each subject dosed with isoniazid (7.5 mg/kg body weight) in the tablet form with water (300ml)

Group B

The subjects in this group were administered isoniazid (500 mg tables) to each dividual after an over night fasting, with water (300 ml) after withdrawing their no tuberculosis treatment one day before the start of study.

Sampling procedures:

All the subjects (Group A & B) were asked to evacuate the bladder before administration of drug. Heparinised blood samples were taken at 0, 1, 2, 3, 4, 5, and 6 hours after drug administration. Plasma was separated by centrifugation at 2000 rpm and stored at -20°C in serum tubes. Similarly, urine samples were collected at 0, 1, 2, and 3 hours, volume of the urine was measured and an aliquot of 5 ml from each sample was stored at -20°C until analysis.

Analysis of free and acetylated isoniazid:

The concentration of free and acetylated isoniazid in the plasma and urine samples, was determined using modified UV method (14), on a Shimadzu UV-240 Graphicard UV-visible spectrophotometer.

Acetylator Phenotyping:

Elimination half life (t 1/2) of the drug was calculated by regression analysis of log of concentrations of blood samples from 2 to 6 hours. The results were compared with those obtained from acetyl-INH/INH ratios study conducted in 0-3 hours blood samples, and acetylation percentage measured in 0-3 hours urine collections. Acetylalion percentage was calculated as follows:

$$\text{Acetylation percentage} \frac{\text{Acetyl-INH}}{\text{Total drug (Fee INH + Acetyl-INH)}} = \text{x } 100$$

Slow and fast acetylators were differentiated accordingly as shown in Table 1 and 2.

Results

Group A

As shown in Table 1, acetyl-INH/NH ratio is 0.73 ± 0.42 for slow acetylators and 1.95 ± 0.53 for fast acetylators. Cases showing this ratio below 1.5 were classified as slow acetylators while those showing above 1.5 were categorised as rapid acetylators. Similar results were obtained by using the plasma half life (t 1/2) as acetylators phenotype determinant. All slow acetylators have half life above 2 hours and 20 minutes and fast acetylators have t 1/2 below 2 hours and 20 minutes. Thus these two methods seem to be reliable for phenotyping of healthy volunteers.

The results obtained by acetylation percentage method (Table 1) indicate that all slow acetylators have acetylation below 50% (mean value 31.01 ± 3.2), while fast acetylators have acetylation above 50% (mean value 66.75 ± 4.21).

According to these three methods, 68 subjects were classified as slow acetylators while 52 as rapid acetylators out of 120 healthy volunteers.

Group B

The results obtained by the three above mentioned methods classify the subjects in this group as slow and fast acetylators and are shown in Table 2. It can be seen that 35 subjects were found as slow and 25 as fast acetylators out of 60 tuberculosis patients.

Discussion

Isoniazid is still widely used to determine acetylator status although dapsone is becoming increasing popular as an alternative drug. Isoniazid is preferred in patients with glucose-6-phosphate dehydrogenase deficiency to avoid haemolysis or in those tuberculosis patients already receiving Isoniazid, as it interferes with the elimination of dapsone (Ahmad et al., 1981). Also, Isoniazid can clearly distinguish slow from fast acetylators while dapsone has been found reliable to accurately phenotype subjects in 2 to 6% cases near the boarder line of phenotype status.

Although the half life method is generally used to phenotype the human subject yet it has the disadvantages of being dependent on volume of distribution (Vd) which may very in different cases and is inversely related to clearance and therefore product of metabolities. Since in fast acetylators, isoniazid undergoes extensive presystemic elimination (Weber and Hein, 1979), half life method would be relatively insensitive towards measuring the acetylation rate. It also requires blood sampling through venous puncturing, a usually painful and inconvenient process.

Although the acetylation ration method (Ellard and Gammon, 1973) is relatively convenient than the half life method (Hutchings and Poutledge, 1986), it requires blood sampling through venous puncturing in addition to being less reliable. In this study it was found that 5 tuberculosis patients who were classified as slow acetylators by the half life and acetylation percentage methods, were not classified as slow acetylators by the acetylation ratio method (Ellard and Gammon, 1973).

In this study the acetylation percentage method is found to be simple, fast and convenient as compared to the previous methods (Hutchings and Poutledge, 1986 Ellard and Gammon, 1973). As urine contains greater concentrations of isoniazid and acetylisoniazid, chances of error are also less and the distinction between slow and fast acetylators is more well defined. It also offers to classify slow and fast acetylators including tuberculosis patients receiving concomitant isoniazid therapy.

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Table 1
Comparision of Acetylator Phenotyping Methods. All values are indicated as means (± SEM) of number of subjects indicated in parenthesis

Subject	Phenotyping method	Fluid	Acetylator Phenotype	
			Slow (n = 68) Acetylator	Fast (n = 52) Acetylator
Healthy Subjects (n = 120)	Half life (hrs)	Plasma	4.2 ± 0.13	2.3 ± 0.08
	Acetyl-INH/INH ratio (3 hrs)	Plasma	0.73 ± 0.42	1.95 ± 0.50
	Acetylation (%) (0-3 hrs)	Urine	31.01 ± 3.2	66.75 ± 4.21

Table 2

	Phenotyping method	Fluid	*Slow (n = 35)	Fast (n = 25)
Tuberculosis Patients (n = 60)	Half life (Hrs)	Plasma	4.30 ± 0.29	2.4 ± 0.12
	Acetyl-INH/INH ratio (3 hrs)	Plasma	1.10 ± 0.75	2.3 ± 0.12
	Acetylation (%) (0-3 hrs)	Urine	35.01 ± 3.60	65.52 ± 3.65

*According to Acetyl-INH/INH ratio slow acetylators are 30 while fast acetylators are 30.

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