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PHARMACOGENETIC STUDY OF THE ACETYLATION PHENOTYPE IN A BULGARIAN POPULATION

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N-acetyltransferase, an enzyme involved in the metabolic inactivation of drugs like isoniazide, some sulfonamides and others is well-known to be under polymorphic genetic control. The acetylation phenotype of the patients may serve as an important guide in foretelling the therapeutic efficacy or tolerability of a particular drug. In the present study we investigated the distribution of the acetylation phenotypes in a group of 100 healthy volunteers of both sexes using sulfadimidine as a substrate. The distribution was found to follow a bimodal pattern, as aspected, with a slight predominance of the "slow" acetylators - in 58 % of the cases, a finding similar to literature data from neighbouring and other European countries. In the men's group the distribution was approximately the same as that in the whole group whilst in the women's one the "rapid" inactivators prevailed. This work represents the first modest attempt in Bulgaria for phenotyping the population according to the individual acetylation status.

Key-words: Genetic polymorphism, N-acetyltransferase, sulfadimidine, "slow" acetylators, "rapid" acetylators, Bulgaria

INTRODUCTION

The interest in the genetically polymorphous biotransformation of drugs, and in particular in the polymorphism of drug acetylation and oxidation, is constantly rising (1 - 3, 5, 6, 9, 13, 14). This interest is determined by the causal relation between polymorphous drug metabolism and some initially puzzling variations in the therapeutic effects of different drugs. This polymorphism is also related to the different individuals' susceptibility in a human population to drugs' adverse effects, as well as to the risk of development of some diseases, including malignancies (2, 7, 13, 14).

According to data from the literature available, the enzyme system responsible for the polymorphic acetylation of drugs like isoniazide, procainamide, depressan, some sulfonamides (e. g., sulfadimidine), as well as carcinogenic aromatic amines, is

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a nonmicrosomic, noninducible N-acetyltransferase (2, 5, 6). The low hepatic acetyltransferase activity is controlled by a single recessive gene. The ratio of "rapid"/"slow" acetylators varies remarkably in different ethnic and/or geographic origin populations. "Slow" acetylators are rarely to be met (5-10 per cent) amongst mongoloid population of the Far East (Eskimos, Japanese, Chinese) and are widely represented (80-90 per cent) in Egyptians or Moroccans, while in the rest of the world their incidence varies between these extremities (2, 4, 5, 11, 12, 14).

The present study aims at two goals: 1. To determine the incidence rate of the acetylator phenotypes in a group of volunteers from the north-east region of Bulgaria using sulfadimidine as a substrate; 2. To compare the results with literature data on the same topic concerning European and other populations.

MATERIAL AND METHODS

The investigation was carried out on 100 healthy volunteers - medical students of both sexes (78 males and 22 females) unrelated to each other with an average age of 22 years. Participants in the study were asked not to consume alcohol and not to take any other medicines at least 48 hours before the experiment. The procedure of the trial was as followed (8, 10):

A tablet of 500 mg sulfadimidine was given to every participant at 7 a. m. with a little of water on an empty stomach. Urine was collected for analysis at about 9 a. m. in bottles provided by the laboratory. It was diluted 1:100 with distilled water. Two tubes with diluted urine and hydrochloric acid added were boiled for 1 hour in a water bath to produce hydrolysis of the acetylated sulfadimidine back to the free form. These two tubes were used for determination of the total sulfadimidine (free + acetylated). Other two tubes of the same content, but not boiled, were used to estimate the free sulfadimidine. The urine was treated with sodium nitrate, ammonium sulfate, and naphthylethylenediamine in a fixed schedule to yield a colour compound. The sulfadimidine concentration was then measured spectrophotometrically at 550 nm. The percentage of sulfadimidine excreted in the acetylated form was given by:

$$A\% = \frac{C_{total} - C_{free}}{C_{free}} \times 100$$

Individuals in which this percentage was higher than 70 were considered "rapid" acetylators but those with a percentage lower than 70 - "slow" acetylators (10).

RESULTS

The frequency distribution of these volunteers according to their acetylator phenotype shows a bimodal curve (Fig. 1).This is considered an evidence for the present genetic control in the activity of the drug-metabolizing N-acetyltransferase.

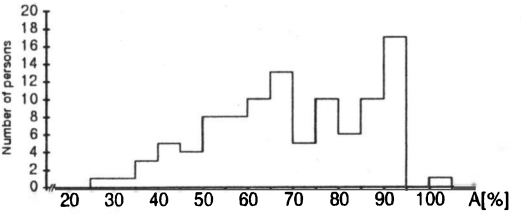

Fig. 1. Frequency distribution histogram of the percentage acetylated sulfadimidine in urine of 100 healthy unrelated volunteers

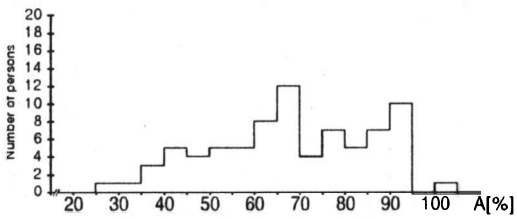

Fig. 2. *Frequency distribution histogram of the percentage acetylated sulfudimidine in urine of 78 healthy male volunteers*

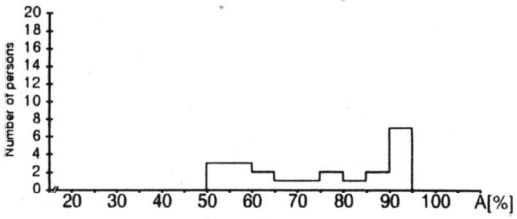

Fig. 3. *Frequency distribution histogram of the percentage acetylated sulfadimidine of 22 healthy female volunteers*

The "slow" acetylators slightly predominate representing 58 per cent of the whole group. From a genetical point of view they are homozygotes. This is probably the reason for the well-shaped "mode" in their grouping.

A certain split in the curve of the group of "rapid" acetylators is to be noted. This fact could be explained by two ways: on the one hand, participants' number in the experiment is relatively small, and, on the other hand, the "rapid" acetylators are not a homogenous group in that they could be either homozygotes (RR), or heterozygotes (Rr) thus some disturbances in the smooth line of the curve could be caused.

The analysis of the frequency distribution of the acetylator phenotypes according to sex reveals that in men it coincides with that of the whole group (Fig. 2). In women, however, a prevalence of the "rapid" acetylators is found out (Fig. 3) - they represent 55 per cent of the cases versus 42 per cent in the whole group.

DISCUSSION

Our results are similar to literature data available concerning the frequency distribution of acetylator phenotypes in some population groups like those of India, Finland, South Norway, the white population of the USA, Hispanic Americans, and Turks. In these groups slow acetylators are established in approximately the same percentage (2, 4, 5, 11, etc.) as in our study. The picture of the Turkish population is of particular interest to us because of the close territorial relations to Bulgaria, although variations are reported according to the different subregions involved in the study (5).

It is generally believed that the acetylation rate depends mainly on the race but not on the gender (5, 13). However, some evidence exists, according to which among female individuals in some species of experimental animals (rats) and in humans "rapid" acetylators are more commonly met (2, 15). A possibility is discussed that the expressiveness of the "rapid" gene may be under estrogen control or that testosteron can be the factor leading to "slowing" of the phenotype in males (2). However, the small number of our female contingent does not allow us to draw a definite conclusion about the observed difference.

The age homogeneity of our group of volunteers prohibits us from discussing on the controversial question about the influence of the age on the acetylation

phenotype. According to certain authors (5, 13), this factor does not exert any effects upon the frequency distribution while other investigators (12) report that among elderly people (e. g., Hungarians) the slow acetylators of sulfadimidine occur more often.

CONCLUSION

We could conclude that as, to our knowledge, no data exist on the pharmacogenetics of the acetylation polymorphism in a Bulgarian population, our investigation may be considered the first modest attempt to fill in this blank space of our medico-biological science.

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Pharmakogenetische Untersuchung des Azetylierungsphenotypen in der bulgarischen Bevölkerung

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Zusammenfassung: Es ist bekannt, daß die N-Azetyltransferase, die die Azetylierung von Isoniazid, einigen Sulfonamiden und anderen Arzneimitteln katalysiert, sich unter einer polymorphen genetischen Kontrolle befindet. Die

Feststellung des Phenotypen der Kranken als "schnnelle" Azetylierer oder als "langsame" Azetylierer könnte zur Prognosierung der therapeutischen Wirksamkeit und der Verträglichkeit entsprechender Arzneimittel dienen. In der vorliegenden Arbeit wurde die Verteilung von 100 Probanden beider Geschlechter nach ihrem azetylierenden Phenotyp hinsichtlich des Sulfadimidins untersucht. Die Ergebnisse stellten eine gut ausgeprägte bimodale Kurve dar. Der Prozentsatz der Langsamazetylierer war leicht überwiegend - in 58 % der Fälle. Diese Daten stimmten mit entsprechenden Angaben von benachbarten Regionen unseres Landes überein. Bei Männern war die Verteilung mit dieser der ganzen Gruppe gleich, während bei Frauen die Schnellazetylierer überwiegend waren. Diese Arbeit stellt einen ersten Versuch für eine pharmakogenetische Phenotypisierung der bulgarischen Bevölkerung nach ihrem azetylierenden Status dar..

Étude pharmacogénétique sur le phénotype acétylant chez la population bulgare

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Résumé: Il est bien connu, que N-acétyltransférase - l'enzyme qui est engagée dans l'acétylation des certains médicaments comme l'isoniaside, quelque sulfonamides et bien autres, se trouve sous un contrôle génétique polymorphique. La connaissance du phénotype acétylant des malades - "rapide" ou "lent", pourrait etre utilisée pour qu'on peut prévoir l'éfficacité du traitement et la tolérance aux médicaments respectifs. Dans ce but on a étudié la distribution d'un groupe de volontaires - 100 personnes des deux sexes, d'après leur phénotype acétylant en utilisant le substrat sulfadimidine. Les résultats forment une courbe bimodale bien marquée. Une prédomination des acétylateurs "lents" - 58 %, était observée, ce qui coïncide avec des données similaires des régions voisines du pays. Chez les hommes la distribution est similaire de celle du groupe entier, tandis que chez les femmes les inactivateurs "rapides" prédominent. Le travail présenté est un premier essai en Bulgarie d'une phénotypisation pharmacogénétique de la population selon son statut acétylant.