EPITHEORESE KLINIKES FARMAKOLOGIAS
KAI FARMAKOKINETIKES, INTERNATIONAL
EDITION 13: 126 (1999)
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Using Caffeine as a Metabolic Probe

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INTRODUCTION

It is known that the N-acetylation of the antituberculosis (TB) drug isoniazid by the liver enzyme N-acetyltransferase is genetically determined. Thus, there are slow and fast acetylators according to the rate of metabolism of the drug. The classification of patients as fast and slow acetylators facilitates the establishment of the appropriate dosage regimen of TB drugs in the rational treatment of TB. Caffeine, which is also biotransformed by N-acetylation, has been widely used as an *in vivo* probe for the assessment of N-acetyltransferase polymorphism. In the present study caffeine was used as a metabolic probe to determine N-acetyltransferase polymorphism in 83 healthy Greek volunteers by means of the molar ratio of 5-acetyl-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (IX) determined in urine following ingestion of caffeine.

METHODS

Urine samples were collected for eight hours following the ingestion of 200 mg caffeine. A 200 ìl acidified urine aliquot was saturated with $(NH_4)_2SO_4$ and 6 ml $CHCl_3$ were added to the sample. The sample was dried under a stream of M_2 at 45 ^oC and resuspended in 200 µl distilled H_2O. Caffeine and its metabolites AFMU and IX were detected by HPLC using a UV detector (λ=280 nm), a nucleosil MN5-C18 (4.6x250 mm) column and 0.05% acetic acid acetonitril (90:10, v/v) as mobile phase. Data acquisition and analysis was performed with the Chrom & Spec software. N-Acetylation phenotype was estimated by the metabolic ratio AFMU/IX.

RESULTS

Frequency distribution analysis of the metabolic ratio AFMU/IX revealed two distinct groups with 66.3% (AFMU/IX=0.75±0.04, n=55) slow acetylators and 33.7% (AFMU/IX=0.75±0.04, n=55) rapid acetylators. Sta-tistical analysis indicated that there is a phenotypic effect (P=0.05). No statistically significant difference was detected between slow and fast acetylators in terms of smoking habits, sex and coffee-drinking habits.

These results are in agreement with previous studies on N-acetyltransferase activity in Caucasians using caffeine as a metabolic probe. They also agree with reports on N-acetyltransferase activity in Greek tuberculosis patients using isoniazid as a metabolic probe. Thus, the use of caffeine as a metabolic probe is a reliable method for the assessment of N-acetyltransferase polymorphism within the Greek ethnic group.