THE CLINICAL APPLICATION OF A NEW SCREENING METHOD FOR THE DETERMINATION OF ACETYLATOR PHENOTYPE USING CAFFEINE

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The N-acetylation of drugs exhibits genetically-controlled polymorphism, characterised by a bimodal frequency distribution in the population of 'slow' and 'fast' acetylators. In clinical practice the determination of acetylator status can aid in the prediction of efficacy and toxicity of N-acetylated drugs, in particular isoniazid and hydralazine (Lunde et al, 1977). The principal objective of this study was to develop and evaluate a rapid, non-invasive screening method using caffeine as the test substance, as suggested by Grant et al (1984), and to compare it with the established sulphadimidine test (Du Souich et al, 1979).

The caffeine and sulphadimidine tests in 26 volunteers were applied to urine and blood samples after administration of a caffeine-containing beverage, either alone or following oral sulphadimidine (10 mg kg^{-1}). Plasma samples were analysed for sulphadimidine before and after hydrolysis, according to a modified Bratton-Marshall spectrophotometric method (Du Souich et al, 1977), and the percentage of acetylated sulphadimidine was determined. The reproducibility of the assay was characterised by relative standard deviation (RSD) values of 9.77% (mean 19.6% acetylation; n = 6), and 1.45% (mean 69.2% acetylation; n = 8), corresponding to 'slow' and 'fast' acetylators respectively. The analysis of caffeine in urine was carried out using a modified HPLC method (Grant et al, 1984), the optimised chromatographic parameters being as follows: 250 x 4.6 mm ID column packed with 5-μm ODS-Hypersil; eluent, 0.05% v/v acetic acid - methanol (90:10, v/v); flow rate, 1.5 ml min^{-1}; detection at 280 nm. The peak height ratio of the two caffeine metabolites, 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (MX), was determined, the RSD of the HPLC assay over 8 days being 6.39% (mean AFMU:MX ratio = 3.44; n = 23).

By the sulphadimidine method two acetylator phenotype groups in the study population were clearly defined, with 17 'slow' and 9 'fast' acetylators, taking the discrimination level as 40% acetylation (Du Souich et al, 1979; Grant et al, 1984). The HPLC method defined the population as comprising 18 'slow' and 8 'fast' acetylators, characterised by a low or a high metabolite peak height ratio, respectively. The upper limit of the ratio for the 'slow' population (mean + 3 SD) was found to be 2.282, while the lower limit for the 'fast' population (mean - 3 SD) was found to be 2.296. The level discriminating between the two groups corresponded to a ratio of 2.300. In this study only one volunteer gave inconsistent results by the two methods.

Urine samples provided by 11 volunteers after the administration of sulphadimidine and caffeine were assayed using the above HPLC method, in order to assess any possible interference in the caffeine test introduced by the other acetylated drug. Although the results showed some changes in the metabolite peak height ratios, in only one case did the apparent acetylator status of a subject change. Dietary caffeine would therefore appear to be an acceptable test substance for the simple, rapid and non-invasive determination of acetylator phenotype based on a single urine sample. The possibility of interference from other acetylated drugs, however, needs to be further investigated.

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