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A rapid method for determination of acetylation phenotype using dapsone

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A rapid, simple one-stage protein precipitation method for the estimation of plasma dapsone (DDS) and monoacetyldapsone (MADDS) concentration by high-pressure liquid chromatography (h.p.l.c.) is described. Its performance in the assignment of acetylator phenotype was compared with a reference h.p.l.c. method utilising an extraction procedure and internal standard. The rapid h.p.l.c. technique combined with measurement of the plasma MADDS/DDS ratio is, in our opinion, the method of choice for the determination of the acetylator phenotype in population studies.

Keywords acetylator phenotype dapsone high-pressure liquid chromatography

Introduction

Several drugs or metabolites undergo acetylation by *N*-acetyl transferase, a non-microsomal (soluble) hepatic enzyme (Drayer & Reidenberg, 1977; Lunde *et al.*, 1977). Acetylation exhibits genetically controlled bimodal distribution within populations: rapid acetylation being dominantly inherited. This has clinical relevance since adverse drug effects are commoner in particular acetylator phenotypes (Lunde *et al.*, 1977). There is also evidence that predisposition to chemically-induced bladder cancer may be linked to acetylation capacity (Cartwright *et al.*, 1982).

Several methods have been reported for acetylator phenotype determination using isoniazid, sulphonamides and procainamide, but these have the disadvantages of multiple sampling, difficult analytical techniques or instability of the compounds measured.

In 1971, Gelber *et al.* determined the ratio of monoacetyldapsone (MADDS) and dapsone (DDS) in plasma following a single dose of DDS and demonstrated that individual acetylation characteristics for dapsone, isoniazid and sulphamethazine were identical. A specific, rapid and sensitive h.p.l.c. method for the determination of DDS, MADDS suitable for rapid allocation of acetylator phenotype in population studies is presented below.

Methods

Standards and reagents

Dapsone (4'4 diamino-diphenyl sulphone) and β-hydroxyethyl-theophylline were obtained from Sigma (Poole, Dorset). Dapsone 100 mg tablets were donated by Antigen Ltd (Roscrea, Eire). Monoacetyldapsone (MADDS) was kindly supplied by Dr A. S. E. Fowle (Wellcome Research, Beckenham, Kent). Other reagents were of analytical grade and used as supplied by BDH Ltd (Poole, Dorset).

Instrumentation

Reversed phase h.p.l.c. was performed using a Pye P4 LC-XPD pump and Rheodyne 70-10 sample injection valve fitted with a 20 μl loop. A C18 Magnusphere column, 15 cm × 4.6 mm, 5 μm average particle size (Magnus Scientific, Sandbach, Cheshire) was used in all studies. The absorbance of the eluent was determined using a Pye Unicam LC-UV variable wavelength ultraviolet detector at 295 nm. Column life may be enhanced by adding a pre-column similar to that used for aspirin (Rumble *et al.*, 1981) to the system.

Acetylation typing

A single 1 ml heparinised blood sample is obtained 2–72 h after a single 100 mg oral dose of DDS. Plasma is separated by centrifugation and stored at −20°C pending analysis.

Rapid method for simultaneous determination of DDS and MADDS in plasma

Plasma (200 μl) was transferred to a plastic 1.5 ml microcentrifuge tube and 20 μl of a 1:1 mixture of perchloric acid (60%) and methanol was added. Samples were vortex mixed for one minute and centrifuged at 9950 g for 2 min. An aliquot (30 μl) of the clear supernatant was injected into the column. The mobile phase consisted of methanol: 0.067 M phosphate buffer pH 5.9 (470:230) supplied at a flow rate of 1.2 ml/min at ambient temperature.

Preparation of standard curves

Plasma standards were prepared by spiking drug-free plasma with known amounts of DDS and MADDS to produce concentrations of 500–2000 ng ml^{-1}. Standard curves were prepared by plotting the peak height of each compound versus their known concentration. Standard solutions of DDS and MADDS were prepared monthly and stored at −20°C.

Reference method

The h.p.l.c. method described by Carr *et al.* (1978) was used as a comparison for the method described above. This technique requires addition of an internal standard, the original method was modified by the use of β-hydroxyethyl theophylline (Hanson *et al.*, 1981). This was followed by alkalinisation, extraction into diethyl ether and evaporation to dryness of an aliquot of the organic phase. Separation was effected on a C18 reverse phase column.

Results

Figure 1 shows typical chromatograms. The retention times for DDS and MADDS were 3.3 and 5.4 min respectively. A single endogenous plasma peak occasionally occurred in some plasmas with a retention time of 4.4 min: this did not interfere with the compounds under investigation.

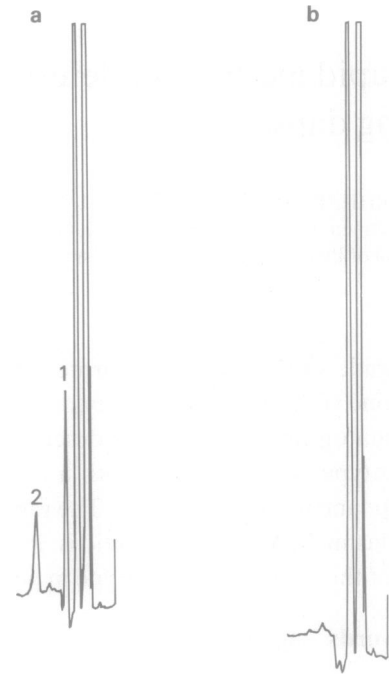

Figure 1 Representative chromatograms of plasma: (**a**) peak 1 dapsone; peak 2 monoacetyldapsone. (**b**) blank plasma from same patient.

Perchloric acid and methanol gave complete protein precipitation and extraction since the standard curves (absolute peak height) of DDS and MADDS from plasma were identical to standard curves for these compounds prepared in water. The linearity of standard curves was excellent ($r > 0.99$) and consistently reproducible. The coefficients of variation (%) for five determinations at 1000, 1500 and 2000 ng ml^{-1} of either compound were 4.7, 6.8, 7.6 for DDS and 2.1, 4.6, 10 for MADDS.

The wavelength of 295 nm used in the present assay gave the optimum detector response for DDS and MADDS. 280 nm (Carr *et al.*, 1978; Zuidema *et al.*, 1980) and 313 nm were investigated with loss of sensitivity. At 295 nm the limits of detection (with 20 μl supernatant) for DDS and MADDS were 60 and 80 ng ml^{-1} respectively.

Using this method at least fifty plasma samples can be analysed by a single operator in a working day.

In a study of repeated estimates of the MADDS/DDS ratio in a single individual the mean ratio was 0.180 with s.d. 0.022 ($n = 19$). The method can also be used for pharmacokinetic studies.

Nineteen plasma samples, taken 2–4 h after

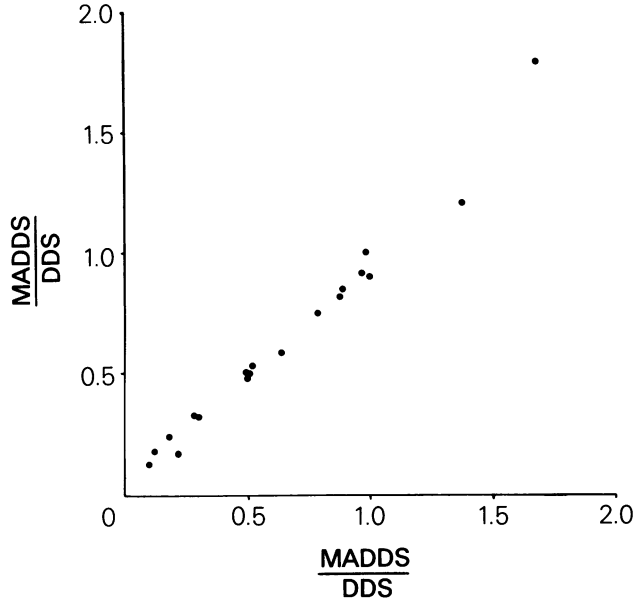

Figure 2 Comparison of 19 MADDS/DDS ratios determined by the rapid protein precipitation method (abscissa) and an extraction method with an internal standard (ordinate).

oral administration of 100 mg DDS to patients with breast cancer were analysed by the rapid protein precipitation method, and the Carr *et al.* (1978) h.p.l.c. method employing extraction and an internal standard. This data is shown in Figure 2. The intraclass correlation coefficient for the paired estimates of the MADDS/DDS ratio (Fleiss, 1975) was 0.98 indicating good conformity of these variates. Analysis of variance gives a variance ratio of method to residual mean squares of 0.477 (on 1 and 18 degrees of freedom) which is not significant and indicates no significant bias in one method.

Discussion

Recent interest in the influence of inherited patterns for the metabolism of foreign compounds in relation to drug toxicity and predisposition to disease has necessitated the use of methods which are simple and rapid yet accurate. For epidemiological work a technique using a single, non-toxic, stable marker drug which requires collection of a single sample, preferably at any convenient time after administration is ideal.

Isoniazid half-life has been widely used to determine acetylator phenotype (Evans & White, 1964) but the test is time-consuming, requires collection of accurately timed plasma samples and employs a high drug dosage. The isoniazid assay is relatively difficult and both isoniazid and its metabolite are unstable in plasma even at −20°C (Hutchings *et al.*, 1982).

Acetylator phenotype can be determined from the ratio of free and acetylated sulphamethazine in a total 8 h urine collection (Evans, 1969) or sulphamethazine half-life (Mattila *et al.*, 1969). Like all such methods the latter required estimation of drug concentration in at least two blood samples using a simple but time-consuming assay. Furthermore, sulphonamides have a relatively high incidence of sensitivity reactions: in population studies it is likely that several individuals would suffer adverse effects from this non-therapeutic administration. The ratio of plasma *N*-acetyl procainamide and procainamide 3–6 h after a dose at steady state can also indicate acetylator status (Reidenberg *et al.*, 1975). This requires only a single plasma sample but multiple procainamide doses are given which could produce adverse effects. Gelber *et al.* (1971) were the first to use the MADDS/DDS ratio for acetylation phenotyping. They demonstrated that individual acetylation characteristics for DDS, isoniazid and sulphamethazine are identical. Reidenberg *et al.* (1973) found that slow acetylators have a plasma MADDS/DDS ratio of less than 0.30 and that this ratio exceeds 0.35 in rapid acetylators. Phenotypic classification by this method agrees with classification using isoniazid elimination half-life, percent urinary excretion of acetylsulphamethazine or the *N*-

acetylprocainamide/procainamide plasma ratio (Reidenberg *et al.*, 1973; Carr *et al.*, 1978; Hanson *et al.*, 1981). As shown by Gelber *et al.* (1971) and confirmed by ourselves (Ahmad & Rogers, 1980) and others, the MADDS/DDS ratio is constant for many hours after a single DDS dose. Using the sensitive assay described, samples may be taken up to at least 96 h after drug administration and the time elapsed since administration is immaterial. This lack of constraint upon sample collection is of great value in field epidemiology. It is also important that adverse effects following a single oral administration of 100 mg DDS are exceedingly rare if individuals with allergy to sulphones or glucose 6-phosphate dehydrogenase deficiency are excluded.

Large epidemiological studies generate large numbers of samples and require easy, rapid methods for sample handling. The MADDS/DDS ratio remains constant for at least a week at room temperature (R. A. Ahmad, personal communication) allowing postal sample delivery.

DDS and MADDS in plasma have been estimated by various methods including the non-specific Bratton Marshall method (Ellard, 1966) and fluorometric methods requiring complex extractions and corrections for overlapping fluorescence spectra (Glazko *et al.*, 1968; Peters *et al.*, 1970). The original h.p.l.c. method of Murray *et al.* (1971) had the disadvantage of long retention times of 40–60 min. Carr *et al.* (1978) described an h.p.l.c. method capable of separating the quantitating MADDS and DDS using monopropionyldapsone as an internal standard which required synthesis and purification by preparative h.p.l.c. Zuidema *et al.* (1980) have described a non-extractive h.p.l.c. method using a slightly more complex sample preparation with perchlorate precipitation followed by potassium carbonate neutralisation. A 500 μl plasma sample is required and separation was made on a reversed-phase column using an acetonitrile-acetic acid mobile phase. As in the present study, an internal standard was found unnecessary. The limit of detection of this assay was not stated. A rapid and accurate thin layer chromatographic method has also been described by Ahmad & Rogers (1981) comparable in speed with the h.p.l.c. method of Carr *et al.* (1978).

A simple, specific, sensitive and rapid h.p.l.c. method for analysis of DDS and MADDS in plasma has been described. This requires only 200 μl plasma and despite the absence of an internal standard yields results comparable with more complex techniques. We advocate the use of DDS as the method of choice for acetylation phenotyping.

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