Urinary D-Glucaric Acid Excretion and Acetanilide Pharmacokinetics before and during Diphenylhydantoin Administration

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Summary. The pharmacokinetics of a single-dose acetanilide (AA) test, and the urinary D-glucaric acid output (UDGAO) were studied in healthy drug free volunteer subjects before and at the end of a fourteen-day period of medication with 5 mg diphenylhydantoin sodium (DPH) per kg metabolically active mass, thrice daily. The steady state plasma concentration (SSPC) of DPH was also determined. The SSPC of DPH was found to vary from 3.4 to 19.6 μg per ml. The mean UDGAO values increased significantly during DPH medication from 9.5 μmol per g creatinine to 29.5 (p<0.002). The half-life of plasma AA concentration decreased significantly during DPH medication from a mean of 4.2 h to a mean of 2.8 (p<0.001). However, the plasma clearance of AA did not change significantly during DPH medication. The increase of UDGAO whilst on treatment with DPH correlated positively with the SSPC of DPH. The evidence from this study suggests that measurement of UDGAO, which is a measure of the activity of the glucuronic pathway does not necessarily indicate the state of induction of oxidizing enzymes in the hepatic endoplasmic reticulum.

Key words: Acetanilide, diphenylhydantoin, D-glucaric acid, induction.

Induction of the enzymes of the hepatic endoplasmic reticulum occurs as a result of exposure to certain drugs and chemicals and may lead to potentially harmful drug interaction effects [1] and ineffective treatment. The normal intrinsic body chemistry may also be affected by microsomal induction, an example being the occasional appearance of rickets and osteomalacia in patients receiving long-continued diphenylhydantoin (DPH) therapy for epilepsy [2]. This sequel is thought to be due to more rapid oxidation and therefore elimination of Vitamin D. On the other hand, enhanced metabolism of bilirubin [3] and cyclophosphamide [4, 5] may be clinically beneficial.

In pharmacogenetic and clinical studies it is of importance to be able clearly to distinguish between environmental and genetic effects. Consequently, it would be useful to have a reliable and accurate method of assessing the extent of induction which is primarily an environmental effect (though the response to it may be to some extent genetically controlled) [6, 7]. Methods presently available include the measurement of urinary 6β hydrocortisol [8, 9] and D-glucaric acid [10].

The studies reported here were performed in an attempt to define the usefulness of urinary D-glucaric acid measurement in relation to the oxidation of acetanilide (AA) and its presumed induction by DPH. AA was chosen as a test drug because it has only one important primary metabolic biotransformation which is para-oxidation to paracetamol; nearly all ingested molecules have a known fate [11], and its pharmacokinetics following a single oral dose are adequately described by a simple one compartment model with exponential absorption and elimination.

Materials and Methods

The 20 subjects were female and male student volunteers aged 20 to 30 years, free of history of drug allergy, current medication during the preceding month, previous hepatic illness or gastrointestinal surgery.

Blood was taken to measure personal plasma blank values before the ingestion of either AA or DPH.

Acetanilide test: Subjects were asked to fast from 9.0 p.m. on the evening before the test. At 9.0 a.m. subjects were observed to ingest a dose of 50 mg AA per kg metabolically active mass (MAM= weight to the power of 0.7; Drabkin, 1959 [12]) with a glass of water. The subjects were then fasted for two hours after which a standard light meal of tea and toast was allowed. Venous blood samples were taken at 5, 7 and 9 h following drug ingestion, anticoagulated with heparin, centrifuged and the plasma stored at −20°C to await analysis.

DPH was given as individually weighed capsules of 5 mg per kg MAM three times daily for 13 days with only one early morning capsule being taken on the 14th day. For the first eleven volunteers venous blood was sampled on the morning and evening of
day 12 and morning of day 13. For the other nine volunteers venous blood was sampled on the evening of day 12 and the morning of day 13. The blood specimens were anticoagulated with heparin, centrifuged and the plasma stored at $-20^\circ$ C to await analysis.

All subjects were given an AA test before starting DPH medication. The first eleven subjects were given a second AA test on day 14 of DPH medication.

An early morning specimen of urine was collected from all subjects firstly on the day of the pre-DPH AA test and secondly on day 14 of DPH medication, i.e. immediately before the second AA test.

DPH plasma levels were measured by the colorimetric method of Dill et al. (1971) [13] with modifications of volumes. The steady state plasma concentration (SSPC) for an individual was the mean of the 2 or 3 observations made on him on days 12 and 13 (after deduction of his personal pre-medication plasma blank value).

AA plasma levels were measured by a modification of the method of Brodie and Axelrod (1948) [11]. DPH was shown not to interfere with the estimation of AA in plasma. Deduction of personal pre-medication plasma blank value was made for all subjects. Previous studies of the decline in plasma AA concentration showed that the 3 time points in an individual provide a reasonable approximation to the result obtained by taking 6 time points.

The plasma elimination rate constant, $k_{el}$, and biologic half-life, $t_{1/2}$, of AA were derived from the regression line calculated by means of a standard least squares technique. The theoretical AA plasma concentration at zero time ($p_0$) was derived by extrapolation of this line; and the apparent volume of distribution $V_D$ calculated as $-D/p_0$ where $D =$ dose of AA. The clearance was taken to be $V_d \cdot k_{el}$.

The statistics of standard lines put through with each determination procedure indicated that there would be only a 1% chance of not detecting a concentration of 1.5 $\mu$g per ml in the case of both drugs.

Urinary D-glucaric acid concentration was measured by the method of Marsh (1963) [14], but using bovine liver $\beta$-glucuronidase (Sigma) instead of rat liver extract. The results are expressed as $\mu$mol D-glucaric acid per g creatinine as recommended by Maxwell et al. (1972) [15]. In the measurement of D-glucaric acid, the concentration effect curve is relatively flat and therefore a source of inaccuracy.

Urinary creatinine concentrations were determined by a standard procedure (Varley, 1962) [16]. Multiple correlations were computed by means of a suitable programme and a digital computer.

Results

Repeatability of DPH Plasma Concentrations

Using a one-way analysis of variance for the 3 plasma concentrations of DPH for the first eleven subjects, $F$ was 47.0 with 10 and 22 degrees of freedom. The intra-class correlation coefficient was 0.91 computed from

$$\frac{B - W}{B + W}$$

where $B =$ sums of squares between individuals and $W =$ sums of squares within individuals (Fisher, 1970) [17]

Using a similar analysis for the 2 plasma DPH concentrations in the other nine subjects $F$ was 10.9 with 8 and 9 degrees of freedom, and the intra-class correlation coefficient was 0.81.

Basic Results

These are summarised in Table 1 and shown in Fig. 1 and 2.

Correlations between Basic Observations and Their Changes

These are summarised in Table 2. A separate computation indicated that there was no correlation between dose of DPH and urinary D-glucaric acid output on DPH.

The more important correlations with their $t$ values and degrees of freedom are given in Table 2. It will be seen that significant relationships have been demonstrated between: —

(i) Change in urinary & urinary glucaric
    glucaric acid output & output on DPH
(ii) AA clearance & AA clearance
    on DPH & pre DPH
(iii) Urinary D-glucaric & SSPC DPH
    acid output on DPH
(iv) Change in D-glucaric & SSPC DPH
    acid output on DPH

Discussion

The main results from the present work can be summarised as follows: —