ASSESSMENT OF PLASMA GLUTAMYL TRANSPEPTIDASE ACTIVITY AND URINARY D-GLUCARIC ACID EXCRETION AS INDICES OF ENZYME INDUCTION

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SUMMARY

1. Urinary D-glucaric acid excretion and plasma γ-glutamyl transpeptidase activity were measured in twenty-five out-patient and eighteen in-patient epileptic children receiving anticonvulsant therapy. Control specimens were obtained from twenty-three children receiving no medication.

2. D-Glucaric acid excretion greater than the control range was present in 88% of in-patients and 52% of out-patients. However, γ-glutamyl transpeptidase activity above the control range was found in 78% of in-patients and only 32% of out-patients.

3. In these forty-three epileptic subjects, there was a significant correlation between D-glucaric acid excretion and plasma γ-glutamyl transpeptidase activity.

Key words: D-glucaric acid, plasma γ-glutamyl transpeptidase, enzyme induction, anticonvulsants.

The influence of hepatic microsomal enzyme induction upon drug and mineral metabolism is now well established (Conney, 1967; Hunter, Maxwell, Stewart, Parsons & Williams, 1971). However, the assessment of hepatic enzyme activity in the individual patient remains difficult because of the lack of suitable methods. Hepatic biopsy with direct determination of enzyme activity is clearly unacceptable for routine clinical use. Of the indirect methods available, estimation of urinary D-glucaric acid excretion, which is dependent upon the activity of a specific hepatic microsomal enzyme system, has been established as a reliable quantitative index of enzyme induction (Hunter, Carrella, Maxwell, Stewart & Williams, 1971). As this method is time-consuming, more readily determined indices have been sought. Hepatic γ-glutamyl transpeptidase (EC 2.3.2.2) is located primarily in the microsomal fraction (Szewczuk, 1966) and recent work has suggested that the presence of elevated plasma γ-glutamyl transpeptidase activity may serve as a useful indirect measure of enzyme induction (Rosalki, Tarlow & Rau, 1971; Whitfield, Moss, Neale, Orme & Breckenridge, 1973).

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The purpose of this study is to compare plasma γ-glutamyl transpeptidase activity and urinary D-glucaric acid excretion in children taking known enzyme-inducing drugs.

**METHODS**

**Subjects**

Forty-three epileptic children, who were coincidently being screened for biochemical rickets, were studied. Eighteen were residential subjects and twenty-five were attending the paediatric out-patient department. There were twenty-six males and seventeen females, aged 3–12 years, all receiving treatment with phenobarbitone alone or in combination with Phenytoin or primidone. The current daily dose of anticonvulsant was recorded, a unit scoring system being used in which 0.13 mmol (30 mg) of phenobarbitone, 0.18 mmol (50 mg) of sodium diphenylhydantoin (Phenytoin) and 0.46 mmol (100 mg) of primidone each equalled 1 unit. Control values were obtained from twenty-three age-matched normal children receiving no medicament. In both epileptic and control children standard liver function tests showed normal results.

**Determination of urinary D-glucaric acid excretion**

An early morning specimen of urine was collected from each subject and stored at −20°C until analysed. Urinary D-glucaric acid was converted into D-glucaro-1,4-lactone by boiling the urine at pH 2. The inhibitory effect of D-glucaro-1,4-lactone upon the release of free phenolphthalein from phenolphthalein glucuronide (Sigma Chemical Co., London) by enzyme hydrolysis (glucuronidase activity of rat liver was used as the source of enzyme) was estimated as described by Marsh (1963). Standard enzyme-inhibition curves were constructed, glucaro-1,4-lactone freshly prepared from D-glucaric acid (Sigma) being used. Urinary creatinine was determined by Technicon Autoanalyser [SMA 6/60 (4+2) Sequence no. 02]. The results were expressed as µmol of D-glucaric acid/g of creatinine. This has previously been shown to correlate well with the 24 h excretion of D-glucaric acid (Hunter et al., 1971).

<p>| Table 1. γ-Glutamyl transpeptidase activity of plasma and urinary D-glucaric acid excretion in control and epileptic children on anticonvulsants |
|-----------------------------------------------|---------------------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
<th>Plasma γ-glutamyl transpeptidase activity (units/l)</th>
<th>D-Glucaric acid excretion (µmol/g of creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls</td>
<td>15.26±0.81</td>
<td>8.60±1.03</td>
</tr>
<tr>
<td></td>
<td>(n = 23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Out-patients</td>
<td>34.12±6.3</td>
<td>36.16±6.2</td>
</tr>
<tr>
<td></td>
<td>(n = 25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>In-patients</td>
<td>51.05±8.49</td>
<td>74.16±10.07</td>
</tr>
<tr>
<td>P</td>
<td>1 &amp; 3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>1 &amp; 2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2 &amp; 3</td>
<td>N.S.</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Mean results (±SEM) are shown with significance of differences. N.S. = not significant.
Determination of plasma $\gamma$-glutamyl transpeptidase activity

A specimen of venous blood was collected from each subject and $\gamma$-glutamyl transpeptidase activity was measured at 37°C by the use of a commercial kit (Boehringer, Mannheim), which employs the kinetic photometric method of Szasz (1969). The coefficient of variation for enzyme estimations was less than 3%. The results were expressed as units/l, i.e. $\mu$mol of substrate transformed min$^{-1}$ l$^{-1}$.

The results of each group were expressed as the mean $\pm$ SEM and analysis was carried out using the Mann-Whitney $u$ non-parametric test and Kendall $t$ rank correlation method.

RESULTS

The mean values of $\gamma$-glutamyl transpeptidase activity and urinary D-glucaric acid excretion of the control, out-patient and in-patient groups are given in Table 1 and the individual values in Fig. 1: 32% of the out-patients and 78% of the in-patients had $\gamma$-glutamyl transpeptidase and epileptic children (OP, out-patients; and plasma $\gamma$-glutamyl transpeptidase activity in controls and epileptic children (OP, out-patients; IP, in-patients) on anticonvulsant therapy.
values above the control range (10–25 units/l). The corresponding values for urinary excretion of D-glucaric acid were 52%, 88% and 1.6–22.8 μmol of D-glucaric acid/g of creatinine.

Fig. 2 shows the relation between plasma γ-glutamyl transpeptidase activity and D-glucaric acid excretion in the forty-three epileptic children. The results were closely correlated ($P < 0.005$).

The mean durations of therapy in the in-patient and out-patient children were 7 years and 3 years respectively, with each group receiving a mean daily dose of 5 units (range 2–9 units). Duration of therapy did not significantly alter D-glucaric acid excretion or γ-glutamyl transpeptidase activity in either group.

**DISCUSSION**

Epileptic children on anticonvulsant therapy have elevated plasma γ-glutamyl transpeptidase activity and urine D-glucaric acid excretion. Both groups, but notably the out-patients, included some patients with normal values. The out-patient children may not have been taking their drugs regularly. This is supported by the finding that only in the in-patients could a significant relation ($n = 18; P < 0.001$) between drug dose and D-glucaric acid excretion be demonstrated. This relation has been shown by other workers (Hunter et al., 1971). Neither group showed a significant relation between plasma γ-glutamyl transpeptidase activity and drug dose.

Treated individuals had a greater proportional increase in D-glucaric acid excretion than in
Indices of enzyme induction

There was also a significant difference between the two treated groups in respect of D-glucaric acid excretion, which was lacking in respect of plasma γ-glutamyl transpeptidase. The enzymes of the glucuronic acid metabolic pathway may be more sensitive to induction by anticonvulsant drugs than is hepatic γ-glutamyl transpeptidase. In one out-patient whose urinary D-glucaric acid excretion rose from 4 to 28.5 μmol/g of creatinine after in-patient anticonvulsant treatment for 1 week, no significant rise in γ-glutamyl transpeptidase occurred (14 units/l to 15 units/l).

Although other workers have shown that elevation of plasma γ-glutamyl transpeptidase activity may accompany accelerated drug metabolism (Whitfield et al., 1973), the activity may arise from enzyme in pulmonary, renal and pancreatic tissue as well as in liver (Szczeklik, Orlowski & Szewczuk, 1961). Elevated activities have been found in cardiac and other disorders (Szczeklik, Szewczuk, Howsad & Kolaczkowska, 1972; Whitfield, Pounder, Neale & Moss, 1972). These other tissues may account for elevated transpeptidase activity with normal or near-normal D-glucaric acid excretion. Thus D-glucaric acid may be both more sensitive and more tissue-specific. However, further studies of plasma γ-glutamyl transpeptidase isoenzymes would be useful.

REFERENCES


