Full Paper

Effect of Antiepileptic Drugs on the Urinary Excretion of Porphyrins in Non-porphyric Subjects

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Abstract. The action of some anticonvulsant drugs as the causal agents of attacks of acute porphyria has been widely documented in the literature. However, little attention has been paid to the effect of these drugs on the urinary excretion of porphyrins in non-porphyric subjects. In a sample of 82 epileptic patients treated with phenobarbital (n = 54), phenytoin (n = 64), carbamazepine (n = 33), and valproate (n = 8), the daily doses were expressed according to a drug score that would reflect the capacity of these drugs as enzymatic inducers when administered in polytherapy. A significantly increased urinary excretion of D-glucaric acid (DGA) and porphyrins was found in this group of patients (P < 0.001), with coproporphyrin being the major fraction in all cases (>60%). Urinary DGA had a highly significant correlation with the drug score (r = 0.783, P < 0.001); however, no significant correlations were found between the urinary porphyrins and DGA (r = 0.005) or the drug score (r = 0.053). Neither was any significant relationship found between the urinary porphyrins and the serum activity of 5'-nucleotidase (r = 0.066) or the presence of a cholestasis objectivized through the presence of the isoform of \( \gamma \)-glutamyltransferase with \( \beta \)-globulins electrophoretic mobility. However, in a group of 10 patients a significant correlation was found between the urinary excretion of porphyrins and \( \beta \)-N-acetylhexosaminidase (r = 0.790, P < 0.01). Therefore, it does not appear that the liver enzyme induction, or even a subclinical cholestasis, produced by the antiepileptic drugs administered to these patients may serve to explain the increase in the urinary excretion of porphyrins. A possible renal origin is proposed for the increase of urinary porphyrins in these cases.

Keywords: antiepileptic drug, urinary porphyrins, liver enzyme induction, cholestasis, renal tubular dysfunction

Introduction

The porphyrias are a group of heterogeneous clinical conditions caused by alterations in the biochemical pathway involved in heme biosynthesis. Many drugs such as phenobarbital are able to induce the cytochrome P-450 with simultaneous alteration of the feedback mechanism of heme biosynthesis, increasing the key enzyme \( \delta \)-aminolevulinic acid synthetase (1). The porphyrinogenic role of anticonvulsant drugs and its implication as precipitating factors for acute porphyrinic attacks have been demonstrated in humans as well as in animal models (2–4). In porphyrinic subjects, these drugs precipitated acute attacks often associated with large increases of the porphyrin precursors \( \delta \)-aminolevulinic acid (ALA) and porphobilinogen (PBG). In non-porphyrinic individuals, the administration of antiepileptic drugs may also produce moderate increases in serum ALA (5) and urinary ALA and PBG (6).

Furthermore, in different reported cases of porphyrias associated with the administration of epileptic drugs, significant increases were found in the urinary excretion of porphyrins, accompanied by a high degree of liver enzyme induction, evaluated using the aminopyrine breath test (3) and the urinary excretion of \( 6\beta \)-hydroxycortisol (4). In non-porphyrinic subjects treated in monotherapy with carbamazepine, McGuire et al. (6) found a significant increase in the urinary excretion of porphyrins and \( 6\beta \)-hydroxycortisol.

The aim of our study was to explore the urinary
excretion of porphyrins in non-porphyrinic epileptic patients treated with anticonvulsant drugs in polytherapy, and its relationship with levels of urinary D-glucaric acid, an indirect test of hepatic enzyme induction (7). Also, in patients treated with antiepileptic drugs, serum concentrations of pyridoxal-5'-phosphate (PLP) appear to be significantly lower than in healthy subjects (8, 9). As the PLP may act as a cofactor of some decarboxylases of porphyrins and the ferrochelatase that take part in the biosynthesis of the heme (10, 11), a study was also made of the possible relationship between the urinary excretion of porphyrins and the enzyme activation of the serum aminotransferases by the addition of PLP to the reaction medium (12). Other aspects considered were the possible influence of cholestasis or renal tubular injury produced by the administered antiepileptic drugs on the urinary excretion of porphyrins in these patients.

**Materials and Methods**

A group of 82 non-porphyrinic epileptic patients (51 male, 31 female), with a mean age (± S.D.) of 36.9 ± 13.4 years, with symptomatic, cryptogenic, or primary generalized epilepsy, without any having had epilepsy secondary to ischemic stroke, were studied. These patients had been in treatment for more than 10 years with phenobarbital (n = 54), phenytoin (n = 64), carbamazepine (n = 33), or valproic acid (n = 8). Therapeutic compliance was adequate in all cases, and no additional pharmacological treatment was given. Blood and urine samples were taken before the morning dose of the anticonvulsant drugs, whose dosage had not been changed in at least the three previous months. As the drugs were administered generally in polytherapy, the daily dose was expressed as units/day according to a drug score in which one unit corresponded to every 30 mg of phenobarbital, 50 mg of phenytoin, and 100 mg of carbamazepine (13, 14). The control group was comprised of 49 clinically healthy individuals (30 male, 19 female) with a mean age of 36.7 ± 11.3 years. All cases of pregnant women or those taking oral contraceptives were excluded. None of the subjects were taking vitamin supplements. The study was approved by the ethical committee of the University of Santiago de Compostela Hospital Clinic, and all participants provided their consent to participate.

The determination of total porphyrins in urine and its electrophoretic separation was carried out according to the method of D’Alessandro Gandolfo and Topi (15) with slight modifications, and the results were expressed as the ratio of porphyrins to urinary concentrations of creatinine (16). Urinary D-glucaric acid (DGA) was determined using an enzymatic procedure (17), and the results were expressed in relation to the urinary creatinine (18). The urinary activities of total β-N-acetylhexosaminidase (Hex, EC 3.2.1.52) and its major isoforms Hex A and Hex B were measured by a previously described procedure (19), and the results were expressed in relation to urinary creatinine. The serum activities of γ-glutamyltransferase (GGT, EC 2.3.2.2), 5'-nucleotidase (5'NU, EC 3.1.3.5), aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), and alcohol dehydrogenase (ADH, EC 1.1.1.1) were determined as previously described (20). Electrophoretic separation of the GGT isoforms was carried out according to the method of Kok et al. (21). The serum activities of AST and ALT were determined with supplementation of PLP to the reaction medium (0.10 mmol/I) according to the recommendations of the Spanish Clinical Biochemistry Society. With the aim of saturating the apoenzyme with PLP and to assure that all possible NADH reactions with substances present in serum were completed (particularly in pyruvate), the reaction mixture was pre-incubated for 2 min before starting the period in which catalytic activity was measured. The serum activities of both aminotransferases were also determined without addition of PLP, and the enzyme activation produced by the coenzyme supplementation was calculated. The urinary α1-microglobulin was determined using an immunonephelometric assay from Dade Behring (Marburg, Germany), and the results were expressed in relation to urinary creatinine.

Statistical analysis of the data was carried out using Microsoft Excel (v.5.0). The Kolmogorov-Smirnov test was applied to check for normality. Parametric tests (Student’s test and Pearson’s correlation coefficient) were used when the data had a Gaussian distribution; otherwise, non-parametric tests (Mann-Whitney test and Spearman’s correlation coefficient) were used. The results were expressed as the mean ± S.D. (median), and the statistical significance was accepted as P-values of less than 0.05.

**Results**

A significantly higher urinary excretion of total porphyrins was found in the patient group than in the control group (Table 1). Figure 1 shows the distribution of the values obtained for the urinary porphyrins in both groups, without any significant differences being found in relation to sex. In the control and patient groups, coproporphyrin was the major fraction (≥60%), and there was no significant difference for the urinary porphyrin pattern between both groups. However, amongst
the patients studied, the excretion of total porphyrins had a significant correlation with the relative proportion in percentage of coproporphyrin ($r = 0.598$, $P < 0.001$), indicating that the increase of total urinary porphyrins in these patients is mainly due to the coproporphyrin.

The urinary excretion of DGA and serum activity of GGT were also significantly increased in the group of patients (Table 2). Although the valproic acid is considered an enzyme inhibitor, the urinary excretion of porphyrins in the group of 8 patients treated with valproic acid ($102.5 \pm 31.7 \mu g/g$ creatinine) was not statistically different from the porphyrin excretion in the remaining 74 patients treated with other antiepileptic drugs ($93.7 \pm 42.7 \mu g/g$ creatinine).

No significant correlation was found between the excretion of porphyrins in the patients studied and urinary DGA ($r = 0.005$), serum GGT activity ($r = 0.168$), or the drug score ($r = 0.053$). Neither did the urinary porphyrins have any significant correlations with the serum activities of 5'NU ($r = 0.066$), AST ($r = 0.088$), ALT ($r = 0.158$), or ADH ($r = 0.048$). In turn, the urinary DGA had highly significant correlations with the drug score ($r = 0.783$, $P < 0.001$) and GGT ($r = 0.364$, $P < 0.001$). Also, the GGT had a highly significant correlation with the drug score ($r = 0.373$, $P < 0.001$). The correlation between DGA excretion and the drug score was significantly higher in male ($r = 0.871$, $P < 0.001$) than in female ($r = 0.671$, $P < 0.001$) patients; however, in none of these subgroups of patients was a significant correlation found between the urinary porphyrins and DGA or drug score (data not shown). No significant correlation was found amongst the patient group between the urinary porphyrins and the enzyme activation of AST ($r = 0.071$) or ALT ($r = 0.182$) on supplementation with PLP. Examination of the urinary

**Table 1.** Urinary excretion of porphyrins and DGA and serum GGT activity in the control and patient groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 49)</th>
<th>Patients (n = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyrins (µg/g creatinine)</td>
<td>62.7 ± 24.8 (61.5)</td>
<td>94.5 ± 41.7 (92.5)***</td>
</tr>
<tr>
<td>DGA (µmol/g creatinine)</td>
<td>18.5 ± 7.2 (16.9)</td>
<td>279.0 ± 210.0 (247.0)***</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>16.9 ± 8.9 (14.0)</td>
<td>70.8 ± 54.1 (52.5)***</td>
</tr>
</tbody>
</table>

Significance: ***/P<0.001.

**Table 2.** Urinary excretion of porphyrins and DGA and some serum enzyme activities in the patients with and without βGGT isoform

<table>
<thead>
<tr>
<th></th>
<th>With βGGT (n = 9)</th>
<th>Without βGGT (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyrins (µg/g creatinine)</td>
<td>97.1 ± 4.3 (96.0)</td>
<td>94.2 ± 41.8 (91.0)</td>
</tr>
<tr>
<td>DGA (µmol/g creatinine)</td>
<td>239.3 ± 111.4 (229.8)</td>
<td>283.9 ± 219.1 (250.0)</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>181.3 ± 49.0 (164.0)***</td>
<td>57.2 ± 36.3 (50.0)</td>
</tr>
<tr>
<td>5'NU (U/l)</td>
<td>10.9 ± 2.5 (11.0)***</td>
<td>7.8 ± 1.8 (8.0)</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>33.1 ± 9.1 (33.0)**</td>
<td>26.1 ± 7.3 (24.0)</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>41.7 ± 11.7 (43.0)**</td>
<td>27.9 ± 14.4 (24.0)</td>
</tr>
<tr>
<td>ADH (U/l)</td>
<td>0.32 ± 0.14 (0.27)*</td>
<td>0.24 ± 0.19 (0.18)</td>
</tr>
<tr>
<td>Drug score (units/day)</td>
<td>9.4 ± 3.6 (10.0)</td>
<td>9.6 ± 5.3 (8.6)</td>
</tr>
</tbody>
</table>

Significance: *$P<0.05$, **$P<0.01$, ***$P<0.001$. 

![Fig. 1. Distribution of the urinary excretion of porphyrins in male (open circles) and female (closed circles) subjects from the control and patient groups.](image)
excretion of uroporphyrin and coproporphyrin did not provide any information with additional interest in any of the cases in relation to that obtained from the excretion of total porphyrins.

From the group of 82 patients studied, in 9 of the cases (11%), an isoform of the serum GGT was found with electrophoretic mobility of the \( \beta \)-globulins (\( \beta \)GGT), which is considered as a sensitive test for cholestasis (22, 23). Table 2 shows the results obtained depending on whether the patients presented the \( \beta \)GGT isoform or not. Despite not finding any significant differences for the urinary excretion of porphyrins and DGA or for the drug score, the activities of GGT, 5'NU, AST, ALT, and ADH were significantly higher in the group of patients who presented the \( \beta \)GGT isoform.

In 10 patients treated with valproic acid (\( n = 5 \)) and carbamazepine (\( n = 6 \)), in which the determination of the Hex activity and \( \alpha \)1-microglobulin urinary excretion was performed, a significant correlation between them was found (\( r = 0.818, P = 0.004 \)). Likewise, the urinary excretion of total porphyrins and Hex showed a significant correlation (see Fig. 2). The correlation coefficients between the urinary excretion of total Hex and coproporphyrin and uroporphyrin were respectively \( r = 0.746 \) (\( P = 0.013 \)) and \( r = 0.566 \) (\( P = 0.088 \)). The correlation of the total porphyrins with isoenzyme Hex A was \( r = 0.839 \) (\( P = 0.002 \)) and with isoenzyme Hex B, \( r = 0.571 \) (\( P = 0.084 \)). The coefficient of correlation between the urinary excretion of porphyrins and \( \alpha \)1-microglobulin was \( r = 0.596 \) (\( P = 0.069 \)).

**Discussion**

In the group of epileptic patients studied, the urinary excretion of porphyrins in 28% of the cases, and DGA in 95% of the cases, was higher than the upper limit of the corresponding ranges of reference. Different authors have shown a significant correlation between the urinary excretion of DGA and the hepatic P-450 cytochrome in humans and experimental animals treated with phenobarbital and phenytoin (24 – 26). The level of enzyme induction produced by antiepileptic drugs is dose-dependent (27), which explains the highly significant correlation found in the group of patients between the urinary DGA and the drug score, which would reflect the enzyme-inducing capacity of the drugs administered in polytherapy (13, 28). The urinary excretion of porphyrins in the group of patients studied did not have a significant correlation with the urinary DGA or the drug score, which does not support the hypothesis proposed by other authors (6) that the liver enzyme induction produced by the administered antiepileptic drugs could explain the increase in urinary porphyrins in non-porphyric subjects.

There is an inverse relationship between the in vivo serum concentration of PLP, the physiologically active form of vitamin B6, and the enzyme activation of serum AST and ALT after supplementation with this coenzyme (12). The fact that no significant correlations were found between the urinary porphyrins and the enzyme activation of both aminotransferases suggests that in the patients studied neither would the excretion of porphyrins be connected with the serum levels of PLP.

In the patients group, serum GGT presented highly significant correlations with the urinary excretion of DGA and the drug score. However, other factors, apart from the actual induction of the hepatic synthesis of the enzyme protein, may involved in the increase of serum GGT activity through the action of these drugs (7, 20). As a result, serum GGT does not appear to be a suitable marker for evaluating liver enzyme induction by antiepileptic drugs (20, 28). Also, in this case, the separation of its enzyme isoforms does not appear to have provided any data of additional practical interest, except for the detection of the \( \beta \)GGT isoform (20), which, as previously mentioned, is considered as a sensitive test for cholestasis (22, 23). In addition, 5'NU is a non-inducible enzyme by phenobarbital-type inducers (29), and the increase of its activity in serum appears to be specific for cholestatic liver injury (30).

There was a dichotomy in the results obtained for the
patients depending on whether or not they presented the βGGT isoform; in the group of 9 patients who presented this isoform the serum activities of both the membrane-bound GGT and 5’NU enzymes (cholestasis markers) as well as the cytosolic enzymes AST, ALT, and ADH (cellular injury markers) were significantly higher than in the group of the other 73 patients without the βGGT isoform. However, no significant differences were found between both groups of patients as regards to the urinary excretion of porphyrins and DGA or the drug score.

In the case of patients with liver diseases, the increase in the urinary excretion of porphyrins (mainly coproporphyrin) appears to depend more on the level of associated cholestasis than on the severity of the hepatocellular damage (31). Consequently, in these patients, the total plasma and urinary porphyrins correlated significantly with the serum biochemical indices of cholestasis as the membrane-bound liver enzymes, but not with the hepatocellular injury markers as the cytosolic enzymes (32). However, in the group of epileptic patients studied, the urinary porphyrins did not present significant correlations with the GGT and 5’NU activities. Furthermore, the existence of cholestasis, at least biochemically detectable through the presence of the βGGT isoform, and possibly produced by the drugs administered, did not significantly affect the urinary excretion of porphyrins.

Many antiepileptic drugs are metabolized to generate reactive metabolites with membrane lipid peroxidation capability, which therefore elicit systemic toxicity (33). The vitamin B6 deficiency causes a decrease in antioxidant defense system and leads to excessive free radical production in liver tissue in rats (34); however, in our patients, the correlation between the urinary excretion of porphyrins and AST and ALT enzyme activation after supplementation with PLP was not statistically significant.

The kidney is not merely a passive filter of non protein-bound plasma porphyrins, but an organ with an important biosynthetic capacity for the heme, mainly localized in the proximal tubules cells (35). The regulation mechanisms of heme synthesis in the kidney are quantitatively similar those seen to operate in liver cells; however, one notable difference is the quantitatively higher level of stimulation that appears to be required to elicit the derepression of renal ALA synthetase (35). In accordance with previously published results (36, 37), an increased urinary porphyrin level appears to be a sensitive marker of the oxidative stress produced by various chemicals in the proximal tubules of the kidney.

The increase of the urinary levels of Hex activity is specific for tubular involvement and should be interpreted as such (38). In epileptic patients, significant increases of urinary α1-microglobulin and Hex activity have been described, suggesting a proximal tubular dysfunction produced by the administered anticonvulsant drugs (39, 40). It is interesting that in our patients in which the determination of the urinary excretion of total Hex activity and porphyrins was performed, a significant correlation between them was found ($r = 0.790$, $P = 0.006$). These results must lead us to consider a possibly renal origin for the urinary porphyrins in non-porphyric patients treated with antiepileptic drugs. We are currently carrying out a study aimed at verifying this hypothesis and its clinical meaning.

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References


