SHORT COMMUNICATION

PLASMA PORPHYRIN CONCENTRATIONS IN PORPHYRIA CUTANEA Tarda

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SUMMARY

1. Plasma porphyrin concentrations in twelve patients with porphyria cutanea tarda (symptomatica) were compared with twenty normal controls.
2. The concentrations in porphyria cutanea tarda were significantly elevated.

Key words: porphyria cutanea tarda, plasma porphyrins.

Porphyria cutanea tarda (symptomatica) (PCT) is an acquired hepatic porphyria, usually caused, in susceptible people, by liver damage from a high ethanol intake. Clinically, patients have skin fragility, hypertrichosis, photosensitivity and hyperpigmentation, and biochemically there is a high urinary excretion of uroporphyrin, hepatic siderosis, abnormal iron metabolism (Turnbull, Baker, Vernon-Roberts & Magnus, 1973) and increased hepatic δ-aminolaevulinic acid (ALA) synthetase (EC 2.3.1.13) (Dowdle, Mustard & Eales, 1967). Venesection, repeated at fortnightly intervals until mild iron-deficiency anaemia occurs, produces a clinical and biochemical remission, which is usually sustained, and is associated with suppression of hepatic ALA synthetase activity (Moore, Turnbull, Barnardo, Beattie, Magnus & Goldberg, 1972).

Photosensitivity in PCT is due to raised levels of porphyrin in the skin (Magnus, 1968). As erythrocyte porphyrin levels are normal, it has been assumed that they are transported from the liver in the plasma, but direct measurements have not previously been reported. This paper deals with the measurement of these plasma porphyrin levels.

MATERIALS AND METHODS

1. Measurement of plasma porphyrin

Ethyl acetate–acetic acid (100 ml; 4 : 1, v/v) was slowly mixed with 25 ml of plasma and either kept at −20°C overnight, or exposed to light and air for 20–30 min, to convert any porphyrinogens into porphyrins (Falk, 1964).
This mixture was passed through an ‘O’ porosity sintered Buchner filter under suction to remove the protein precipitate, which was then washed with two 5 ml volumes of ethyl acetate-acetic acid and sucked dry.

_Uroporphyrin._ Most of the uroporphyrin is retained with the protein precipitate, but in order to remove remaining quantities from the filtrate, the ethyl acetate-acetic acid mixture was extracted with 25 ml aliquots of saturated sodium acetate, in a separating funnel, until the extracts ceased to show fluorescence under ultraviolet light (wavelength <365 nm).

The protein precipitate in the Buchner funnel was mixed with 25 ml of aq. ammonia (1 mol/l) and the ammonia solution collected under suction. This was repeated until the extracts, after acidification with HCl (1·37 mol/l), ceased to show fluorescence.

The aq. ammonia and sodium acetate extracts were combined and brought to pH 1·5 by the addition of conc. HCl. Uroporphyrin was extracted, from 50 ml of the mixture, using 2 x 25 ml of redistilled cyclohexanone. If a protein precipitate formed between the layers it was removed, washed with HCl (1·37 mol/l) and the washings added to the final acid extract (vide infra). Diethyl ether (100 ml) was added to the cyclohexanone and uroporphyrin extracted into HCl (1·37 mol/l) and quantitated by spectrophotometry at the Soret maximum of 406 nm.

_Coproporphyrin and protoporphyrin._ After removal of the uroporphyrin the ethyl acetate-acetic acid phase was washed with 25 ml of sodium acetate (0·22 mol/l) and the porphyrin taken into HCl (4·11 mol/l). The ethyl acetate-acetic acid was then discarded. Solid sodium acetate was added to the acid until it was neutral to Congo Red. The porphyrin was then extracted with 2 x 25 ml of diethyl ether. Coproporphyrin was measured by extraction from the ether phase with HCl (0·1 mol/l) and read at the Soret maximum of 400 nm, and protoporphyrin by extraction into HCl (1·37 mol/l) and read at 407 nm.

All porphyrins were identified by paper chromatography of the free porphyrins with 2·6 lutidine and then by cellulose acetate electrophoresis in barbitone buffer (50 mmol/l; pH 6·8).

_Calculation._ The equation used was:

\[
\frac{[2E_{\text{max}} - (E_{430} + E_{380})]}{V} = \frac{F_n}{V} = \text{nmol of porphyrin/l of plasma}
\]

where n is the volume of acid extract in ml; V is the volume of plasma taken in ml; F is the factor 1·001 for uroporphyrin, 1·278 for coproporphyrin and 2·178 for protoporphyrin. The \(E\) values are the extinctions at the given value in nm. The \(F\) factors are modified from the values given by Rimington (1961) to give the result in molar values.

Urinary, faecal and blood porphyrins were measured by the method of Rimington (1961) and the normal values are taken from Moore (1970). ALA and porphobilinogen (PBG) were measured by the method of Mauzerall & Granick (1956) and X porphyrin by the method of Rimington, Lockwood & Belcher (1968) as described by Moore, Thompson & Goldberg (1972). In Table 1 the values for X porphyrin are given as µg/g of faeces as the molecular weight is unknown.

2. _Patients_

These were a group of ten men and two women seen at Edinburgh Royal Infirmary and diagnosed on clinical and biochemical evidence as having PCT. Eleven had histories of moderate to excessive ethanol ingestion, the twelfth (S.C.) had not, and the cause of her PCT is unknown. Plasma was taken from the blood at their first therapeutic venesection.
TABLE 1. Porphyrin precursors and porphyrins in porphyria cutanea tarda (symptomatica)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>In urine (μmol/24 h)</th>
<th>In faeces</th>
<th>In erythrocyte (nmol/l)</th>
<th>In plasma (mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALA</td>
<td>PBG</td>
<td>URO</td>
<td>COPRO</td>
</tr>
<tr>
<td>J.H.</td>
<td>M</td>
<td>60</td>
<td>0.51</td>
<td>0.45</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>S.C.</td>
<td>F</td>
<td>75</td>
<td>0.58</td>
<td>0.57</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>J.B.</td>
<td>M</td>
<td>51</td>
<td>0.51</td>
<td>0.26</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td>W.D.</td>
<td>M</td>
<td>67</td>
<td>0.58</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>G.P.</td>
<td>M</td>
<td>57</td>
<td>0.58</td>
<td>0.25</td>
<td>0.20</td>
<td>0.12</td>
</tr>
<tr>
<td>W.R.</td>
<td>M</td>
<td>73</td>
<td>0.39</td>
<td>0.17</td>
<td>0.02</td>
<td>0.22</td>
</tr>
<tr>
<td>J.C.</td>
<td>M</td>
<td>64</td>
<td>0.37</td>
<td>0.09</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>W.W.</td>
<td>M</td>
<td>41</td>
<td>0.58</td>
<td>0.13</td>
<td>0.14</td>
<td>0.95</td>
</tr>
<tr>
<td>A.W.</td>
<td>M</td>
<td>47</td>
<td>0.48</td>
<td>0.05</td>
<td>0.07</td>
<td>0.57</td>
</tr>
<tr>
<td>R.A.</td>
<td>M</td>
<td>62</td>
<td>0.71</td>
<td>0.58</td>
<td>0.52</td>
<td>0.13</td>
</tr>
<tr>
<td>E.N.</td>
<td>F</td>
<td>52</td>
<td>1.71</td>
<td>1.14</td>
<td>1.13</td>
<td>264</td>
</tr>
<tr>
<td>D.M.</td>
<td>M</td>
<td>68</td>
<td>0.15</td>
<td>0.05</td>
<td>0.03</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Normal range: 0-40.5 0-15.9 0-0.05 0-0.43 0-0.08 0-0.2 0-15 0-64 0-657 0-1.6 0-0.14 0-3.0

Abbreviations: ALA = δ-aminolaevulinic acid; PBG = porphobilinogen; URO = uroporphyrin; COPRO = coproporphyrin; PROTO = protoporphyrin; X = X porphyrin.
3. Control subjects

These were twenty healthy blood donors, age and sex matched to the patients, whose erythrocytes were being used for packed-cell transfusion, and whose plasma would otherwise have been discarded.

RESULTS

The porphyrin measurements in the twelve patients (Table 1) show the abnormalities typical of PCT (Goldberg, 1971). The urinary uroporphyrin excretion was very high with some elevation of urinary coproporphyrin excretion. There was a constant elevation of faecal X porphyrin but faecal copro- and proto-porphyrin excretions were only variably raised. There was no rise in urinary δ-aminolaevulinic acid or porphobilinogen excretion or in the concentrations of erythrocyte porphyrins. There was a marked and invariable rise in all three plasma porphyrin concentrations, especially of uroporphyrin, which, in one patient, rose as high as 5559.5 nmol/l.

DISCUSSION

In PCT, in addition to the urinary and faecal porphyrin abnormalities, the plasma levels of porphyrin are extremely high. As erythrocyte porphyrin levels are normal, it is in the plasma that the porphyrins pass from the liver to the skin. As remission occurs, the plasma levels drop (M. R. Moore, G. G. Thompson, B. R. Allen, J. A. A. Hunter & S. Parker, unpublished observations). Since the usual method of treatment is venesection, which provides a large volume of plasma, the estimation of plasma porphyrin seems to offer a convenient way of assessing the effects of therapy, avoiding the inaccuracy and inconvenience of 24 h urine collections.

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REFERENCES


