Bullous dermatosis of end-stage renal disease: a possible association between abnormal porphyrin metabolism and aluminium

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Abstract

Background. Bullous dermatosis (BD) is becoming increasingly recognized in patients with end-stage renal disease (ESRD). It is clinically reminiscent of porphyria cutanea tarda, but its detailed pathogenesis remains unclear. Studies have shown increased porphyrin levels in dialysis patients, and this may partly explain the skin lesions and photosensitivity evident in these patients. In experimental studies, aluminium can induce various abnormalities in porphyrin and haem metabolism. This study investigated a possible involvement of porphyrin metabolism and aluminium in the development of bullous dermatosis in chronically dialysed patients.

Methods. Three groups were studied (12 healthy controls; 12 patients on chronic dialysis without BD and six patients on chronic dialysis with BD). Clinical characteristics of these patients were evaluated and the levels of plasma porphyrins, erythrocyte porphyrins and enzymes involved in the porphyrin chain were determined.

Results. The patients with BD were predominantly male, 50% had ADPKD, all had been on dialysis for a long period of time (7.8 ± 2.1 years) and all were anuric. CAPD and haemodialysis were used equally in the affected patients. Aminolaevulinic dehydratase activity was significantly reduced in all ESRD patients (892 ± 47 versus 302 ± 36 versus 408 ± 37 nmol/ml RBC/h). Plasma uroporphyrins as well as RBC protoporphyrin were significantly elevated in ESRD patients (1.7 ± 0.6 versus 21.6 ± 4.7 versus 43.4 ± 12.0 nmol/l) and (1.43 ± 0.14 versus 2.4 ± 0.42 versus 4.19 ± 2.44 μmol/l) respectively. Serum Al levels were markedly elevated in patients with BD (28.3 ± 10.0 μg/l). Both uroporphyrin and protoporphyrin were significantly more elevated in ESRD patients with BD compared to ESRD patients without BD.

Conclusions. Elevated plasma porphyrin levels in ESRD patients are caused by lack of urinary excretion and the inability of haemodialysis and CAPD therapy to remove them. These elevated porphyrin levels may lead to the development of porphyria cutanea tarda symptoms. Elevations in plasma uroporphyrin, red blood cell protoporphyrin, and elevated Al levels suggest a possible relationship between an Al 'load' and abnormal porphyrin metabolism in the development of overt skin disease in the dialysed patient.

Key words: porphyrins; bullous dermatosis; porphyria cutanea tarda; aluminium, end-stage renal failure; dialysis

Introduction

The development of bullous dermatosis (BD) in patients with end-stage renal disease (ESRD) has been well described [1–5], and has been shown to be both clinically and histologically similar to porphyria cutanea tarda (PCT) [5–7]. The pathogenesis of PCT has been attributed to a decreased activity of uroporphyrinogen decarboxylase (UROD) [8], which leads to an accumulation of highly carboxylated porphyrins. The abnormal UROD activity results from an inherited autosomal dominant trait or is acquired by some subjects when exposed to certain precipitating factors such as iron overload, alcohol abuse or oestrogen therapy [6]. Although decreased UROD activity was recently reported in haemodialysis patients [9], the pathogenesis of BD in patients with ESRD remains unclear. Conflicting results concerning the level of plasma porphyrins in ESRD patients with BD have been reported. Normal values were demonstrated in several cases [1–4], but increased porphyrin levels were shown in others [5,6,8], and it has been suggested that these high levels of plasma porphyrins could contribute to the development of BD in ESRD patients [6,8]. Increased erythrocryte coproporphyrin and protoporphyrin levels have also been described in ESRD, and these could also play a role in the BD of ESRD patients [10–13].

Elevated serum aluminium (Al) may be a further precipitating factor in the development of BD in patients with ESRD [2]. Al has been shown to influence
bacterial porphyrin biosynthesis [14]. In rats, administration of Al led to the induction of experimental porphyria [15,16]. In haemodialysed patients a correlation between the level of aluminium and the concentration of RBC protoporphyrin has been reported [11,17]. Finally, King et al. [18] have described a dialysis patient who developed overt PCT after being exposed to a high Al concentration in the dialysis water supply.

The aim of this study was, therefore, to determine if a possible association exists between BD, the impairment of porphyrin metabolism, and serum Al levels in patients with ESRD. Plasma and RBC porphyrins, serum Al and the activities of various enzymes of the haem biosynthetic pathway were determined in a group of healthy controls and two groups of ESRD patients, with and without bullous dermatosis.

Subjects and methods

Subjects

Seven patients with BD were identified among our dialysis population. BD was diagnosed by the attending nephrologist (UG, AK) with the help of a consulting dermatologist. These seven patients were included in the study based on the following criteria:

(a) bullous dermatosis, primarily on the dorsal surface of the hands, with spontaneous rupture and ultimate scar tissue formation;
(b) mechanical fragility of photo-exposed skin;
(c) long periods of latency interspersed by acute attacks of blister formation.

Exclusion criteria included:

(a) alcoholism;
(b) malnutrition;
(c) diabetes mellitus, a possible cause for BD;
(d) the long-term use of medications known to cause BD (frusemide, alphamethyldopa, clomidine, oestrogens, tricyclic antidepressants, phenytoin, tetracyclines, sulphas drugs or anti-tuberculosis agents);
(e) the use of ‘tanning’ beds or prolonged exposure to sunlight.

No histopathology or immunofluorescence examinations were performed on the patients.

Blood samples were taken from all patients during an ‘acute’ period of blister attacks.

Two additional groups, matched for age and sex, were investigated. These included 12 ESRD patients without BD; six on haemodialysis and six on CAPD, and 12 healthy volunteers. Informed consent was obtained from all the participating subjects.

Methods

Serum Al was determined by the inductive coupled plasma method.

Quantification of porphyrins

Porphyrins were determined by an HPLC method.

Apparatus. The HPLC apparatus consisted of an HP 1090L solvent-delivery system (Hewlett-Packard, Avondale, PA), equipped with a Rheodyne (Cotati, CA) 7010 injector and a 100-μl external loop. A C18 reversed-phase column (100 × 4.6 mm i.d., HP Hypersil octadecylsilane, 5 μm particles) was used. Fluorometric determination was performed by a programmable fluorescence detector (HR 1046A) at 404 nm excitation and 615 nm emission wavelengths. Calculations were carried out by an HP-3393 computing integrator.

Samples and standard preparations

Plasma. The dimethyl sulphoxide (DMSO) method described by Gebri et al. [12] was used with slight modifications. Briefly, 1 ml plasma was mixed vigorously for 1 min with 0.36 ml DMSO and then 0.15 ml of TCA 50% was added. The mixture was then centrifuged at 4°C, 1500 g, and 100 μl of the supernatant were injected into the HPLC system.

Blood. Porphyrins of whole blood were extracted according to the method described by Piomelli [19]. The acidic extract (1.5 mol/L HCl) was concentrated by evaporation, centrifuged (3000 g, 10 min) and injected into the HPLC system.

Standards. A porphyrin acids marker kit containing 10 nmol each of 8, 7, 6, 5, 4 and 2 (meso)-carboxylic porphyrins was dissolved in 2 ml of 3 mol/L HCl (5 μmol/l) and used as a stock solution. Prior to HPLC analysis, 5 μmol/l protoporphyrin was prepared separately. Both standard solutions were mixed at a ratio of 1:1, diluted in 1 mol/L HCl, centrifuged and administered into the system.

Separation procedure. The method of Lim and Peters [20] was modified for our system [21]. The mobile phases were (a) 100 ml/l acetonitrile in methanol and (b) 100 ml/l acetonitrile in 1 mol/l ammonium acetate, pH 5.1. The elution plan was linear gradient from 100% B to 35% B for 30 min, followed by 12 min of linear gradient from 35% B to 10% B, 5 min of isocratic elution, and an additional 5 min for returning to 100% B, at a flow rate of 1 ml/min.

Determination of enzyme activities

Aminolaevulinic acid dehydratase (ALAD) [EC 4.3.1.24] and porphobilinogen deaminase (PBGD) [EC 4.3.1.8] were determined according to the methods described by Del et al. [22] and Magnusen et al. [23] respectively.

UROD activity was estimated by the method previously described [9] which was based on the methods of McManus et al. [24] and Luo & Lim [25].

Materials. Porphyrin acids marker kit and pentacarboxyl porphyrin I were obtained from Porphyrin Products (Logan, UT, USA). Sodium and mercury were purchased from Aldrich (Milwaukee, WI, USA) and the solvents for HPLC, acetonitrile and chromatographic grade methanol, were obtained from Merck (Darmstadt, Germany). 5-aminovaleric acid and porphobilinogen were obtained from Sigma Chemical Co. (St Louis, MO). All other reagents were of the highest purity available.

Statistical analysis

Data are presented as mean ± SE. Comparisons between the groups were carried out either by parametric tests (one way
analysis of variance and students t test) or by a non-parametric test (Mann-Whitney) according to the normal or skewed distribution of results. Two-tailed $P < 0.05$ was considered significant.

**Results**

Seven patients with BD (5.2% of our dialysis population) were identified. One patient was not available for the study, and, therefore, the ESRD + BD group consisted of six patients. Clinical and biochemical data are given in Table 1. Five patients with BD were men, and one was a woman. This male gender tendency did not reach statistical significance. The primary renal disease was ADPKD in three patients. Two patients had chronic glomerulonephritis, and one had adult medullary cystic disease. Three subjects were administered in-patient haemodialysis and the other three were CAPD patients. All patients were anuric. The ESRD patients with BD had received dialysis therapy for a significantly longer period of time compared to those patients without BD (7.8 ± 2.1 years versus 2.6 ± 0.4 years). The haemoglobin concentration was higher in those patients with BD, but there was no difference in serum ferritin levels. Two patients with BD were hepatitis C virus carriers, a difference from those ESRD patients without BD which is statistically insignificant (Table 1).

The results of several haem biosynthetic pathway parameters are given in Table 2. The plasma uroporphyrin (URO) level was increased in both groups with ESRD compared to healthy controls. ESRD patients with BD had a significantly higher concentration of uroporphyrin in their plasma compared to ESRD patients without BD. Coproporphyrin (COPRO) and protoporphyrin (PROTO) concentrations in RBC were increased in both groups with ESRD, when compared to the healthy controls, but they did not differ from each other. The activity of ALAD in both groups of patients with ESRD was less than 50% of its activity in healthy controls. The activities of PBGD and UROD in RBC of both groups of patients with ESRD were not significantly different from their activities in the controls.

Al concentration in the sera of patients with ESRD is illustrated in Figure 1. Al was substantially higher in ESRD patients with BD than in ESRD patients without BD (28.3 ± 10.0 versus 6.7 ± 0.9 µg/l, $P < 0.05$). Normal serum aluminium levels ranged from 0 to 3 µg/l in the control group with intact renal function.

**Discussion**

ESRD related BD occurred in more than 5% of our patient population. Clinically, the patient presents with multiple bullae on the extensor surface of the hands. On rupture, these bullae can cause pain and are susceptible to secondary infection. The disease process can undergo spontaneous remissions and exacerbations. The term pseudoPCT has been used to describe these patients with BD, because most of them do not manifest the associated hypertrichosis or sclerodermoid features of true PCT [26], and secondly, because no abnormality in porphyrin metabolism is observed [1,2,4]. However, true hereditary PCT has been documented in at least one dialysis patient. This patient

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**Table 1. Clinical and biochemical data on ESRD patients with and without BD**

<table>
<thead>
<tr>
<th></th>
<th>ESRD</th>
<th>ESRD + BD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>2/10</td>
<td>1/5</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.5 ± 3.1</td>
<td>50.4 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Haemodialysis/CAPD</td>
<td>6/6</td>
<td>3/3</td>
<td>NS</td>
</tr>
<tr>
<td>Period on dialysis (years)</td>
<td>2.6 ± 0.4</td>
<td>7.8 ± 2.1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.1 ± 0.2</td>
<td>12.0 ± 0.6</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>101.8 ± 28.3</td>
<td>61.7 ± 15.8</td>
<td>NS</td>
</tr>
<tr>
<td>HCV positive*</td>
<td>1/12</td>
<td>2/6</td>
<td>NS**</td>
</tr>
</tbody>
</table>

* Hepatitis C virus positive by polymerase chain reaction (PCR).
** Fisher’s exact probability test.

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**Table 2. Haem pathway parameters of controls and ESRD patients with or without bullous dermatosis**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ESRD</th>
<th>ESRD + BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>12</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Plasma porphyrins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>URO</td>
<td>1.7 ± 0.6</td>
<td>21.6 ± 4.7</td>
<td>43.4 ± 12.0</td>
</tr>
<tr>
<td>RBC porphyrins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>µmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COPRO</td>
<td>0.10 ± 0.02</td>
<td>0.34 ± 0.09</td>
<td>1.02 ± 0.47</td>
</tr>
<tr>
<td>PROTO</td>
<td>1.43 ± 0.14</td>
<td>2.40 ± 0.42</td>
<td>4.19 ± 2.44</td>
</tr>
<tr>
<td>Enzymes*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALAD</td>
<td>892 ± 47</td>
<td>302 ± 36</td>
<td>408 ± 37</td>
</tr>
<tr>
<td>PBGD</td>
<td>35.9 ± 1.2</td>
<td>34.4 ± 2.5</td>
<td>34.4 ± 2.9</td>
</tr>
<tr>
<td>UROD</td>
<td>14.8 ± 1.0</td>
<td>10.6 ± 1.1</td>
<td>10.5 ± 3.2</td>
</tr>
</tbody>
</table>

* Enzyme activities are expressed as nmol product/ml RBC/h.

P < 0.01 vs control. * P < 0.05 vs ESRD.

URO, uroporphyrin; COPRO, coproporphyrin; PROTO, protoporphyrin; ALAD, aminolaevulinic dehydratase; PBGD, porphobilinogen deaminase; UROD, uroporphyrinogen decarboxylase.
developed widespread cutaneous lesions at a late stage, 2 years after beginning haemodialysis [27].

Based on the small sample of patients studied, some preliminary statements regarding the clinical features of ESRD-related BD can be made. Most of the patients were men, similar to the male preponderance of PCT [28], and a high percentage of ADPKD was found in these patients. Interestingly, both PCT and adult polycystic kidney disease are inborn errors of metabolism, inherited via an autosomal dominant trait. Affected patients have been on dialysis for a substantial period of time (a mean of nearly 8 years), and all are anuric. Patients on CAPD were affected as much as patients on haemodialysis.

In both groups of patients with ESRD, an accumulation of uroporphyrin in plasma, coproporphyrin and protoporphyrin in RBC, and a reduction in ALAD activity were observed. In addition, an elevation in serum Al was demonstrated. These findings are in agreement with previous studies [10,11,13,29]. A decrease in ALAD activity, similar in magnitude to that documented here, has been previously described in both dialysed and non-dialysed uremic patients [6,10,13]. It may be that some ALAD is produced in the renal cortex and, therefore, ALAD activity will be reduced in patients with severe impairment in renal function [6,13]. Alternatively, ALAD activity in uremics may be inhibited by 'uremic serum factors' [10,13]. The observed higher level of ALAD activity in the ESRD + BD group compared to the ESRD without BD group is an interesting finding, and may be due to the ability of Al to induce ALAD activity [30]. However, the enzyme activity in the ESRD + BD group remained significantly lower than in the controls. Therefore, the low ALAD activity in ESRD cannot explain the BD seen in these patients. However, ESRD patients with BD differed from ESRD patients without BD with respect to three biochemical parameters which may predispose them to the development of skin lesions; namely, plasma uroporphyrin, RBC protoporphyrin and serum Al concentration. All these parameters were significantly higher in those dialysed patients with bullous dermatosis.

The plasma uroporphyrin concentration in ESRD patients with BD approached plasma uroporphyrin concentrations found in patients with PCT and actually surpasses them in several cases; a finding previously reported by Day et al. [6]. Non-uraemic patients with PCT excrete large quantities of highly carboxylated porphyrins in the urine, but they are retained in the plasma of the anuric ESRD patients on dialysis for two reasons. Firstly, there is no urinary excretion of these porphyrins, and secondly, there is a failure of standard haemodialysis procedures to remove URO [31]. This situation can produce dramatic increments in plasma porphyrin levels. Therefore, although porphyrin plasma levels in ESRD patients with BD sometimes resemble that of 'true' PCT, the elevated levels are due to impairment of renal porphyrin excretion and not because of uroporphyrin overproduction secondary to a partial UROD deficiency as in the patient with PCT. However, irrespective of the mechanisms leading to the increased plasma porphyrin concentration, the elevated level may result in skin photosensitivity and thus contribute to the development of BD similar to PCT [6,8]. In PCT it is well accepted that the accumulation of plasma uroporphyrin is partly due to a deficiency in UROD [7]. In this study, UROD activity measured in ESRD patients with, or without BD, was not statistically different from that of controls. In addition, the activity of UROD in the ESRD + BD group did not differ from that of the ESRD group without BD. This suggests that RBC UROD does not play a major role in the appearance of BD in ESRD.

Another possible cause for BD in ESRD patients is a high RBC protoporphyrin level. These high levels, documented by others, have now been confirmed in this study [32,33]. The major cause for this high protoporphyrin level is reduced ferrochelatase activity. This enzymatic deficiency is classically responsible for erythropoietic protoporphyria, a disease in which skin lesions are present. By et al. [17] have also shown a direct relationship between high Al levels and high protoporphyrin levels in haemodialysed patients.

Al interferes with haem biosynthesis. Following oral administration of Al, rats expressed a marked increase in the activity of haem oxygenase, the rate-limiting enzyme in haem catabolism [29]. Al can also induce an increased porphyrin synthesis [6] and can cause a significant elevation in the activity of aminolaevulinate synthase (ALAS), the rate-limiting enzyme of heme biosynthesis [29]. Furthermore, in-vitro and in-vivo studies in rats have described the activation of ALAD by Al [34]. In bacteria, Al led to a 65% reduction in intracellular haem and a fivefold increase in accumulation of porphyrins [14]. Finally, intraperitoneal administration of Al in rats leads to an abnormal excretion of porphyrins in the urine, resembling the pattern seen in PCT, and this effect was more pronounced in partially nephrectomized rats [16]. This last study implies that the precipitating factors in PCT may possibly include Al, especially in a renal failure setting.

A possible association between Al and ESRD-related BD has been described in humans. After 7 years on haemodialysis, a man developed skin lesions which were histologically compatible with PCT. He had elevated plasma porphyrins. The overt PCT developed after 8 months of home dialysis and the use of water with high Al concentration. All skin lesions subsided clinically and plasma porphyrin levels returned to normal when the softened water was replaced by deionized water [18]. Another study described three haemodialysis patients with Al toxicity and bullous dermatosis. Following long-term treatment with desferoxamine, the skin manifestations in all three patients resolved [35]. Two other studies describe the successful therapy of haemodialysis related PCT with desferroxamine. Desferroxamine chelates both iron and aluminium, and therefore Al removal could have played a role in the success of the therapy [36,37]. Finally, Yasuda et al. described a 37-year-old woman on haemodialysis for 7 years, who developed PCT-like
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symptoms. The patient’s skin lesions resolved several months after the cessation of aluminium hydroxide [38].

In conclusion, the high serum Al concentration in ESRD patients may affect certain enzymes in the haem biosynthetic pathway leading to an overproduction and accumulation of porphyrins, principally uroporphyrin and protoporphyrin. This, together with the reduction in removal of porphyrins from the plasma in patients with a very low or non-existent GFR, may partially explain the formation of BD in the ESRD population.

References

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