PLATELET FUNCTION IS ENHANCED, NOT DEPRESSED IN URAEMIA

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Summary

Platelets from undialysed chronic renal failure patients (mean plasma creatinine 1120μmol/L) were studied using turbidometric aggregometry and different agonists (arachidonic acid (AA), ADP, collagen, ristocetin and calcium ionophore) and were more aggregable than normal controls to low doses of agonists and with lower thresholds compared with normal controls. Platelets from chronic haemodialysis patients were also more aggregable to AA compared with normals, though aggregation to other agonists was normal or depressed. Plasma fibrinogen was elevated in both chronic renal failure and haemodialysis patients. Differential responses to agonists and agonist doses indicate that several agonists and doses should be used in studying uraemic platelets. Hyper-aggregable platelets and elevated fibrinogen, together with hypertension and hyper-lipidaemia may account for the high cardiovascular mortality of haemodialysis patients.

Introduction

Patients with chronic renal failure have a prolonged skin capillary bleeding time, and an increased tendency to haemorrhage. They paradoxically also show an increased incidence of atheroma and thrombosis with high cardiovascular mortality. Since platelets are involved both in haemostasis and in the pathogenesis of atheroma, the assessment of platelet function in uraemia is important. Platelet function studies in uraemia using turbidometric aggregometry have reported varying results. Platelet function in undialysed uraemic patients has been reported to be depressed [1], though such a defect of platelet function would be expected to protect against atheroma. Haemodialysis may fully [1], partially [2] or fail to correct [3] this defect. Platelet function may also be enhanced in haemodialysis patients [4].

Review of these studies reveals variation in methodology which may contribute to the differences in results obtained, in that different aggregating
agents in a variety of concentrations have been employed, and there is lack of standardization of platelet counts and citrate anticoagulant concentration. We have shown that correction of citrate concentration is essential when studying uraemic platelets whose haematocrits may vary widely [5]. Uraemic platelet function may appear depressed or enhanced depending on the dose of agonist used [6].

We have therefore compared uraemic platelet function using turbidometric aggregation in undialysed chronic renal failure patients, haemodialysis patients and healthy controls using a variety of agonists and agonist doses, with standardization of platelet rich plasma citrate concentration and platelet count. In addition certain coagulation factors and platelet-release proteins have been measured, since abnormalities of these may also reflect a disturbance of homeostasis in uraemia.

Patients and methods

The following groups were studied: 1) 14 undialysed chronic renal failure patients (plasma creatinine 499–2176 µmol/L, mean 1120 µmol/L), due to hypertension (4), obstructive uropathy (4), chronic pyelo/interstitial nephritis (3), polycystic kidneys (1), chronic glomerulonephritis (1), unknown (1); 2) 14 patients on chronic haemodialysis, receiving 8–22.5 m² hours/week (mean 14.5 m² hours/week) dialysis with cuprophan dialysers; 3) 18 healthy controls. Diabetic or nephrotic patients, those with low platelet counts and those receiving drugs known to affect platelet function had been excluded. Blood for platelet aggregation was taken into 3.13% trisodium citrate anticoagulant. Samples from haemodialysis patients were taken pre-dialysis. Citrate concentration was corrected to a concentration that would be present in blood of a haematocrit of 0.55 [5]. Platelet rich plasma was produced by centrifugation at room temperature for 15 minutes at 150G and also at 1500G for 10 minutes to produce platelet poor plasma. Platelet poor plasma was used to correct platelet count to 200 x 10⁹/L. Platelet rich plasma was kept at 37°C in stoppered tubes. Aggregation was performed in an optical aggregometer (Albert Brown, Leicester, UK). Arachidonic acid (AA) (Sigma), collagen (Hormon Chemie) and ADP (Sigma), each with four concentrations, and also ristocetin (Lundbeck) and calcium ionophore A23187 (Sigma) were used as agonists. Aggregation was measured as a percentage of the optical transmission of platelet poor plasma; rate was determined by a tangential line to the slope of the aggregation curve (%/min), and maximum aggregation was the maximum amplitude (%). Threshold for aggregation to AA and ADP (secondary aggregation) was also determined. Each agonist and dose was used in the same sequence to minimize the effects of variation in platelet response with time, aggregation being completed within 150 minutes.

Plasma concentrations of fibrinogen (Claus technique), Factor VIIIc (two-stage assay, Diagen), Factor VIII-related antigen (VIII RAg) (radial immunodiffusion, Hoechst) and antithrombin III (ATIII) (chromogenic assay, Kabi) were measured. As a marker of platelet α-granule release, plasma concentrations of β-thromboglobulin (Amersham) and Platelet Factor 4 (Abbott) were measured.
by radioimmunoassay, from blood taken into EDTA/theophylline/PGE₁ anticoagulant at 4°C. Results are expressed as mean ± standard error of mean and comparison between groups is by unpaired two-tailed Student’s ‘t’ tests.

Results

Platelet aggregation

Chronic renal failure compared with normals  Uraemic platelets were markedly more aggregable to lower doses of AA (Figure 1a), with an increased sensitivity to AA as shown by a lower threshold (Figure 1d). Maximum aggregation to low

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Figure 1a,b. Platelet aggregation (mean ± SEM) in chronic renal failure and haemodialysis platelets compared with normal controls
Figure 1c–g. Platelet aggregation (mean ± SEM) in chronic renal failure and haemodialysis platelets compared with normal controls.

dose collagen was also increased (Figure 1b). Threshold for secondary aggregation to ADP was lower (Figure 1e), and ristocetin aggregation was enhanced (Figure 1f). However, aggregation to higher doses of agonists was normal or depressed (ADP 5μM max, Figure 1c).

*Haemodialysis compared with normals* Aggregation of platelets from haemodialysis patients to lower doses of AA was again enhanced with a lower threshold
(Figure 1d). ADP threshold was lower than normals but now not significantly (Figure 1e). Maximum aggregation to higher collagen doses was, however, now depressed (1μg/ml 61.3±3.2%, 2μg/ml 63.6±3.3, p<0.02). Ristocetin aggregation was now similar to normals (Figure 1f) and maximum aggregation to calcium ionophore was significantly less than normals (Figure 1g).

**Haemodialysis compared with chronic renal failure** With ristocetin, platelets from haemodialysis patients were less aggregable than those from uraemic patients (p<0.05) (Figure 1f), although with other agonists differences were not significant.

**Coagulation factors**

Fibrinogen, Factors VIII RAg and VIIIc levels in chronic renal failure and haemodialysis patients were twice that of normals. ATIII levels were the same as normals (Table I).

**TABLE I. Coagulation factors and platelet release proteins in chronic renal failure and haemodialysis patients and normal controls (mean ± SEM, range)**

<table>
<thead>
<tr>
<th></th>
<th>Chronic renal failure (n=14)</th>
<th>Normals (n=18)</th>
<th>Haemodialysis (n=14)</th>
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<tbody>
<tr>
<td><strong>Factor VIIIc (U/ml)</strong></td>
<td>2.15±0.18 (0.70–3.36)</td>
<td>1.01±0.07 (0.59–1.53)</td>
<td>2.12±0.18 (0.85–3.28)</td>
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<td>p&lt;0.001</td>
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<td><strong>Factor VIII RAg (U/ml)</strong></td>
<td>2.51±0.23 (1.11–4.62)</td>
<td>1.15±0.08 (0.64–1.68)</td>
<td>2.37±0.31 (0.87–4.53)</td>
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<td>p&lt;0.001</td>
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<td><strong>ATIII (%)</strong></td>
<td>97.6±3.3 (61–118)</td>
<td>97.4±2.9 (67–115)</td>
<td>94.7±4.8 (55–123)</td>
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<td><strong>Fibrinogen (g/L)</strong></td>
<td>4.85±0.43 (3.22–8.90)</td>
<td>2.39±0.12 (1.00–3.30)</td>
<td>4.31±0.32 (2.56–7.20)</td>
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<td>p&lt;0.001</td>
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<tr>
<td><strong>β-thromboglobulin (ng/ml)</strong></td>
<td>121±14 (93–191)</td>
<td>43±3 (17–69)</td>
<td>170±13 (74–232)</td>
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<td>p&lt;0.001</td>
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<tr>
<td><strong>Platelet Factor 4 (ng/ml)</strong></td>
<td>12.9±2.1 (2–26)</td>
<td>6.4±0.7 (0.4–12.4)</td>
<td>18.3±3.1 (1.5–35.5)</td>
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<td></td>
<td>p&lt;0.01</td>
<td>p&lt;0.001</td>
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Chronic renal failure compared with haemodialysis: all not significant differences except β-thromboglobulin: p<0.02
Platelet release proteins

Plasma β-thromboglobulin and Platelet Factor 4 levels were elevated in chronic renal failure and haemodialysis patients compared with normals (Table I).

Discussion

Platelet function has previously been reported to be depressed in chronic renal failure [1], and may or may not be corrected by haemodialysis [1-4]. We have demonstrated, using standardized platelet count and citrate concentration, that undialysed chronic renal failure patients are in fact more aggregable (‘hyper-aggregable’) than normal controls to low-dose AA and collagen and to ristocetin. Threshold for aggregation is lower to both ADP and AA and as such chronic renal failure patients are ‘trigger-happy’. However at high agonist doses, chronic renal failure platelets are normal or, in the case of high-dose ADP, depressed. Unlike Remuzzi et al [6] we were unable to demonstrate decreased aggregability at high doses of AA. Although haemodialysis platelets were also more aggregable to AA, aggregation to other agonists was normal or depressed. This suggests that, in contrast to previous studies, haemodialysis has a tendency to depress platelet function, although when platelets from chronic renal failure patients are compared directly with haemodialysis patients, there is statistical significance only with ristocetin. This probably reflects varying plasma urea and creatinine values, length of uraemia and dialysis regime in the two groups. This standardized method should allow these studies to reflect intrinsic function of platelets in these groups, although in an individual patient high or low platelet counts may increase or decrease respectively the overall contribution of platelets in haemostasis. If the differential response of platelets to different agonists and concentrations reflects physiological influences on platelets, this may also in part explain the paradoxical association of bleeding and thrombosis in uraemia. When aggregometry is used to investigate uraemic platelet function, a range of agonists and concentrations should therefore be employed.

The observation of depressed platelet function in uraemia has been used to explain the observed bleeding tendency. This bleeding tendency may be reversed by correction of anaemia by transfusion [7] or by DDAVP [8] (which may raise Factor VIII levels and alter multimer pattern). It is possible that anaemia, abnormal Factor VIII function, a plasma inhibitor of platelet/Factor VIII interaction [9], and enhanced prostacyclin production from vascular endothelium could then account for the bleeding tendency, while hyperaggregable platelets in undialysed chronic renal failure patients may contribute to the thrombotic tendency. In addition, we have shown that fibrinogen levels are elevated in both undialysed and dialysed patients which has been shown to be a risk factor for atherogenesis and may contribute to platelet hyperaggregability. Elevated VIII RAg levels could partly reflect chronic endothelial injury. Raised plasma Factor 4 levels suggests increased platelet activation in uraemia, although elevation of β-thromboglobulin levels also results from decreased renal clearance.

Hypertension has been isolated as a risk factor for cardiovascular mortality in several studies, and hypertension existing prior to the commence of dialysis
[10] may be particularly important, as might lipid abnormalities [10]. It is possible, therefore, that the atheroma and cardiovascular mortality of haemodialysis patients may result from contributions from hyperaggregable platelets, elevated fibrinogen, hypertension and lipid abnormalities. Anti-platelet therapy might therefore be considered early in progressive renal failure in combination with strict control of hypertension and lipid-lowering therapy.

References