PLATELET ABNORMALITIES IN RENAL TRANSPLANTATION

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Summary

Forty-six transplant patients had lower platelet serotonin and higher plasma β-thromboglobulin (β-TG) than normal controls. These abnormalities were more pronounced in acute rejection (AR) than in chronic rejection (CR), but were also present in normally functioning transplants (FT). Patients also showed lower serum levels of thromboxane B₂ (TxB₂) than controls. Plasma fibrinopeptide A (FPA) was higher in AR, but not in CR and in FT, than in controls.

Therefore, in renal transplant recipients, platelets continue to circulate after in vivo activation, the abnormalities being roughly proportional to the extent of graft injury. Granule-bound substances secreted into the circulation might produce ischaemia and platelet aggregates, damaging the graft and aggravating the rejection lesions.

Introduction

An ‘acquired’ storage pool depletion has been reported in several renal diseases [1, 2], including transplant rejection [2]. This abnormality might have potential for tissue injury because granule-bound substances after secretion recruit additional platelets to the haemostatic plug and produce vasoconstriction.

To assess the extent of platelet stimulation we investigated the platelet release reaction in a large number of renal transplant recipients, some with AR others with CR or with FT. We also evaluated the serum levels of TxB₂, a stable metabolite of thromboxane A₂, and plasma FPA, a fragment specifically cleaved from fibrinogen by thrombin.

Patients

Forty-six recipients of kidney allografts were investigated.

Acute rejection In 13 patients the study was performed after the clinical diagnosis of AR was made. The diagnosis was based on an increase in plasma creatinine
concentration by at least 30 per cent over baseline values. Increased body temperature, tenderness over the graft and proteinuria were regarded as supportive of the diagnosis. Blood samples for investigation were taken just before anti-rejection IV methylprednisolone pulse therapy was started. In these patients the mean levels of plasma creatinine concentration were 3.7 ± 4.9mg/dl.

Chronic rejection In 15 patients, chronic rejection was diagnosed on the basis of slowly progressive, irreversible increase in plasma creatinine concentration after several months to a value at least 30 per cent above the patients’ previous baseline levels. Extensive investigations excluded causes of late transplant insufficiency other than chronic rejection. Patients were investigated between 88 and 320 weeks (mean 197 weeks) after transplantation. Mean plasma creatinine was 3.6 ± 3.2mg/dl at the time of examination.

Normally functioning transplants Eighteen patients were studied between 94 and 344 weeks (mean 187 weeks) after transplantation at a time when function was stable for at least one year and plasma creatinine was normal (1.0 ± 0.4mg/dl).

Normal controls Age-matched hospital personnel served as controls.

Methods

Plasma $\beta$-thromboglobulin was measured on platelet-poor plasma using the reagent and following the instruction of a commercial kit (Radiochemical Centre, Amersham, UK). Plasma fibrinopeptide A was determined on platelet-poor plasma by the radio-immunoassay technique of Nossel, as modified by Kochum [3]. Trasylol (1000 KIE/ml) and heparin (1000 IU/ml) in 1.5M NaCl were added to the blood sample in 1 : 19 proportion by volume. Analysis kit (IMCO Corporation, Stockholm, Sweden) was used. Platelet serotonin was measured on platelet-rich plasma by a fluorimetric method [4]. Serum thromboxane $B_2$ was measured in the supernatant serum of blood clotted with thrombin by radioimmunoassay [5], using a specific antiserum.

Statistical analysis All the values are given as mean ± 1SD. The significance of the differences between groups was tested with the non-parametric Mann-Whitney $U$ test. Spearman’s rank correlation test for non-normally distributed values was employed to evaluate correlations.

Results

The results are summarised in Table I.

Plasma $\beta$-TG was significantly higher in patients with AR ($p < 0.01$), and, to a lesser extent, CR ($p < 0.05$) and FT ($p < 0.05$) than in normal controls. In no instance were $\beta$-TG levels correlated with those of platelet serotonin or plasma creatinine, the highest Spearman’s correlation coefficient being found between $\beta$-TG and creatinine in AR ($r = 0.50$, $p > 0.05$).

Platelet serotonin was significantly low in all groups of renal allograft recipients, but the difference from normal controls was less marked in the FT group ($p < 0.01$)
TABLE I. Mean ± 1SD values of plasma beta-thromboglobulin, platelet serotonin, serum thromboxane B$_2$ and plasma fibrinopeptide A in normal controls, functioning transplants, chronic rejection, and acute rejection. $P$ values for comparison between changes in normal controls and each group of transplant patients

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Functioning transplants</th>
<th>Chronic rejection</th>
<th>Acute rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma beta-thromboglobulin (ng/ml)</td>
<td>16 ± 7</td>
<td>39 ± 33</td>
<td>72 ± 49</td>
<td>85 ± 37</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>Platelet serotonin (ng/10$^4$ platelets)</td>
<td>0.34 ± 0.08</td>
<td>0.27 ± 0.08</td>
<td>0.18 ± 0.88</td>
<td>0.13 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Serum thromboxane B$_2$ (pmol/10$^4$ platelets)</td>
<td>441 ± 155</td>
<td>262 ± 170</td>
<td>260 ± 180</td>
<td>324 ± 241</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.01</td>
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<tr>
<td>Plasma fibrinopeptide A (ng/ml)</td>
<td>1.5 ± 0.8</td>
<td>1.2 ± 0.9</td>
<td>1.9 ± 1.1</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>p = NS</td>
<td>p = NS</td>
<td>p = NS</td>
<td>p &lt; 0.01</td>
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than in the CR ($p < 0.001$) or the AR ($p < 0.001$). There was no significant correlation between plasma serotonin and plasma creatinine ($r = 0.09$, $p > 0.05$).

Serum TxB$_2$ was significantly lower than in normal controls in all groups of transplant patients ($p < 0.01$). There was no significant correlation with platelet serotonin, plasma β-TG, and plasma creatinine.

Plasma FPA was not elevated in CR and in FT groups, but in the AR group plasma FPA levels were significantly higher than in controls ($p < 0.01$). FPA was not significantly correlated with platelet serotonin, plasma β-TG or serum TxB$_2$. It showed a positive correlation with plasma creatinine ($r = 0.62$; $p < 0.05$) only in AR patients.

Discussion

It is apparent from previous studies that platelets are involved in AR and, to a lesser extent, in CR [2, 6]. This study demonstrated platelet abnormalities not only in those instances, but also in normal kidney transplants.

In all groups of patients we found an increase in plasma β-TG, a platelet-specific protein normally contained in the alpha-granules. Simultaneous measurements showed that platelet serotonin levels were lower in transplant patients than in controls. Metabolically inactive storage of serotonin is contained in electron-dense delta-granules. The substance is extruded whenever platelets aggregate and undergo secretion. Elevated plasma β-TG and low platelet serotonin levels show that platelet activation does occur, and demonstrate that partially empty, ‘exhausted’ platelets can circulate in transplant recipients. The degree of storage
pool depletion was higher in AR than in CR. The same abnormality was also present in FT.

The synthesis of prostaglandins, endoperoxides and thromboxane A₂ is necessary for platelet aggregation and secretion in response to agonist substances. Thromboxane A₂ is an extremely potent but labile substance, able to induce platelet aggregation and secretion as well as vasoconstriction. TxB₂, its stable metabolite, may be considered a measure of the maximum amount of thromboxane A₂ produced by platelets. Low serum levels were found in transplant patients, indicating an impairment of platelet arachidonic acid metabolism. This could reflect depletion or inhibition of substances and/or enzymes as a result of their in vivo challenge by the inducers of the release reaction.

The stimuli for platelet activation are probably different in AR, CR, and FT. Thrombin is a powerful trigger of the release reaction. To evaluate the possible role of this enzyme in inducing platelet abnormalities, we measured the serum levels of FPA, an indirect index of thrombin generation. Confirming previous studies [3], we found elevated values of FPA in AR, while the levels were normal in CR and FT. This could reflect an aggressive immunological attack directed at the endothelial cell histocompatibility antigens during AR. Exposure of basement membrane collagen and microfibrils would lead to platelet aggregation and thrombin generation, which in turn can stimulate platelet activation. However, the lack of correlation between plasma FPA and platelet serotonin or plasma β-TG would indicate that thrombin, although important, is not the only trigger of platelet activation in AR. Other mechanisms — such as circulating immune-complexes [7], platelet autoantibodies [8], proteases from the damaged kidney, and platelet activating factor which is released from sensitised basophils and from platelets themselves — might (co)operate in inducing the release reaction. The same mechanisms might be responsible for the abnormalities found in CR and in FT, where the immunological aggression is less severe.

The occurrence of acquired storage pool depletion in renal transplant recipients might have clinical consequences. Platelets release a factor that promotes proliferation of endothelial and myointimal cells [9] and facilitates, the development of oblitative vascular lesions in the graft. Moreover the secretion of serotonin and thromboxane A₂ from platelets induces ischaemia and platelet aggregation which may contribute to microvascular blockade and functional impairment of human kidney transplants.

References

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4 Drummond AH, Gordon JL. *Thromb Diath Haemorrh 1974; 31: 266*
6 Mowbray JF, Paryanda A. *Thromb Diath Haemorrh 1971; Suppl 45: 183*
8 Landis Th F, von Felten A, Berchtold H. *Acta Haemat 1979; 61: 2*
Open Discussion

RITZ (Heidelberg) The platelet data that you obtained with adequate methodology were ascribed by you to in vivo platelet activation presumably within the graft. I would like to raise another possibility — the effect of uraemia itself. Several authors, e.g. Cameron, showed low platelet serotonin in uraemia, and other authors, e.g. Niewietowsky, showed abnormal release reaction. Could you comment on this alternative explanation?

PONTICELLI This is a very important point. I am familiar with the papers showing that there is a relationship between plasma beta-thromboglobulin and plasma creatinine in glomerulonephritis and in severe cases of renal insufficiency. We looked for this possible correlation using the Spearman’s rank correlation test which is a very sensitive test, but we couldn’t find any correlation between plasma β-TG and creatinine. Moreover, we found elevated levels of beta-thromboglobulin also in normally functioning transplants. Therefore, I conclude that although we cannot completely rule out a role for renal insufficiency, in our experience the rise of levels of β-TG are more related to platelet activation than to renal insufficiency. I think that it is possible to say the same for intra-platelet serotonin content. Some investigators found low levels of serotonin in renal insufficiency but only in patients with severe renal insufficiency, while in our patients the mean plasma creatinine was about 3.7mg/dl. Moreover, we couldn’t find a relationship between platelet serotonin and plasma creatinine in our patients.