THROMBOXANE B₂ AND β₂-MICROGLOBULIN AS EARLY INDICATORS OF RENAL ALLOGRAFT REJECTION

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Summary

In a prospective study the diagnostic value of urinary thromboxane B₂ (TXB₂) and β₂-microglobulin (βMG) in renal allograft rejection was studied in 34 patients after transplantation. Twenty-four episodes of rejection were diagnosed by clinical symptoms. The clinical diagnosis of rejection was confirmed by an increase of urinary TXB₂ in 21 (88%) cases. The augmented renal excretion of TXB₂ preceded the clinical signs of rejection for 2.0 ± 0.75 days. The symptoms in the remaining three (12%) cases of supposed allograft rejection without increased urinary TXB₂ were caused by non-immunological events (urinary tract infection, acute tubular necrosis).

No elevated TXB₂ excretion was observed during urinary tract infection, sepsis, and acute tubular necrosis whereas urinary βMG increased during these events as during transplant rejection.

Urinary TXB₂ was found to be an early, specific, and sensitive marker of renal allograft rejection with greater reliability than βMG excretion or clinical signs of rejection.

Introduction

The early diagnosis of renal allograft rejection is thought to have a better prognosis if the therapy is initiated immediately [1]. As the clinical diagnosis of transplant rejection is difficult, especially under immunosuppressive therapy with cyclosporine, a number of laboratory tests were developed to facilitate the early institution of therapy. The activation of cellular blood components and of platelets during allograft rejection is associated with an increased intrarenal formation of arachidonic acid metabolites. The renal excretion of TXB₂, the stable hydrolysis product of the arachidonic acid metabolite TXA₂ is thought to be an indicator of transplant rejection [2].

The present study was undertaken to investigate the diagnostic value of TXB₂ as specific marker of renal allograft rejection in comparison with clinical symptoms and the urinary excretion of βMG [3].
Patients and methods

Thirty-four consecutive patients (16 females and 18 males, mean age 35.7 ± 1.8 years, age range 16–54 years) were studied from the day of transplantation to day 20–51 (mean observation time: 30.3 ± 2.2 days). All patients received a cadaver kidney.

Immunosuppressive therapy consisted of cyclosporine (Cys) and low dose steroids in 26 patients. The dosage of Cys was chosen according to the European Multicenter Trial [4]. Eight patients were treated by conventional immunosuppressive therapy with azathioprine (A) (4mg/kg/day initially, stepwise reduction to 2.5mg/kg/day or less depending on the peripheral white blood cell count) and prednisolone (P) (2mg/kg/day on day one to 12, reduction to 25mg/day within four weeks after transplantation).

Rejection was diagnosed clinically by the presence of one or more of the following symptoms: rise in body temperature, graft tenderness of the kidney transplant, decreased urine output and endogenous creatinine clearance, ultrasonic increase of allograft size and decreased allograft perfusion on radionuclide scanning. The therapy for rejection consisted of prednisolone 500mg/day IV (Cys group) or 1000mg/day IV (A plus P group) every second day for three to six days.

Immunoreactive TXB₂ in 24hr urines collected on indomethacin (8–16μg/ml urine) was determined directly by a specific and sensitive radioimmunoassay previously described by our group [5]. For daily analysis the incubation procedure was modified. The incubation time was shortened to 120 minutes at a temperature of 28°C. The lower limit of detection (10% inhibition of binding label to the antiplasma) was 2pg/ml, 50 per cent displacement of binding of label from the antiplasma was caused by 110pg TXB₂.

βMG in urine was determined by a commercially available radioimmunoassay (Phadebas β₂-micro test, Pharmacia Diagnostics, Uppsala, Sweden).

Daily urinary TXB₂ and βMG concentration were compared with the 24 hour urine output, endogenous creatinine clearance, and clinical symptoms of rejection as described above. Values are given as mean ± SEM, Student’s ‘t’ test for paired or unpaired observations was used for statistical analysis.

Results

Thirty-four patients were studied for a period of 146 weeks after renal transplantation. No deaths occurred during this time. Differences in graft survival, serum creatinine concentration and rejection rate between group I (Cys) and group II (A plus P) were not significant (Table 1).

Twenty-four rejection episodes were diagnosed according to clinical symptoms. The diagnosis of rejection was confirmed by an increase of urinary TXB₂ in 21 (88%) out of 24 cases. In the absence of rejection urinary TXB₂ concentration did not exceed 0.30ng/ml (mean 0.18 ± 0.09ng/ml, n = 27). During rejection TXB₂ increased to 0.88 ± 0.17ng/ml (n = 21, p<0.001), 2.0 ± 0.75 days before the clinical diagnosis of allograft rejection. The symptoms in three (12%) further supposed rejection episodes were caused by urinary tract infection in two and by acute tubular necrosis in one case.
TABLE I. Outcome of renal transplantation in 34 patients treated with cyclosporine (Cys) or azathioprine plus prednisolone (A+P)

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients (n)</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Graft Survival (%)</th>
<th>Serum Creatinine (μmol/L)</th>
<th>Rejection (episodes/patient)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Cys)</td>
<td>26</td>
<td>♂ 18, ♀ 14</td>
<td>35.4 ± 2.2 (16 - 50)</td>
<td>81</td>
<td>188 ± 15 (106 - 318)</td>
<td>0.73</td>
</tr>
<tr>
<td>II (A+P)</td>
<td>8</td>
<td>♂ 6, ♀ 2</td>
<td>36.4 ± 3.3 (26 - 54)</td>
<td>75</td>
<td>198 ± 19 (106 - 336)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Mean ± SEM (range)

An increase of urinary TXB₂ was observed in a further five cases without conspicuous clinical symptoms. Three episodes of elevated TXB₂ concentration were observed during the first 10 days after transplantation and coincided with a prolonged improvement in allograft function, the remaining two episodes of elevated urinary TXB₂ were observed during times of slightly increased body temperature supposedly attributable to urinary tract infections. In all five cases urinary TXB₂ levels decreased and graft function improved within three to six days without specific rejection therapy. No correlation existed between TXB₂ urine concentrations and Cys blood levels determined by radioimmunoassay (y = 0.422 + 8.841 × 10⁻⁵ x, r = 0.051, n = 337).

Urinary βMG concentration increased in all cases of clinical diagnosis of rejection from below 5.0μg/ml (mean 3.24 ± 0.76μg/ml, n = 25) in the absence of rejection, urinary tract infection, systemic infection or acute tubular necrosis to 14.70 ± 3.73μg/ml (n = 21, p<0.005), 0.14 ± 0.63 days after the clinical diagnosis of transplant rejection. The values of urinary βMG and TXB₂ during allograft rejection in relation to the time of diagnosis of rejection are summarised in Table II.

An increase of βMG urine concentrations was also observed in eight (73%) out of 11 episodes of urinary tract infection, in two cases of sepsis and one case of acute tubular necrosis. During the initial phase after transplantation urinary βMG exceeded the basal level (3.24 ± 0.76μg/ml, n = 25) in all primary functioning allografts (initial βMG urine concentration 25.3 ± 5.1μg/ml, p <0.001).

The radioimmunological determination of TXB₂ in urine was highly reproducible (intra-assay variation 5.8%, interassay variation 7.2%), no degradation of TXB₂ occurred in relation to urine temperature (≤37°C) and pH (3 to 10) whereas βMG was rapidly degraded in urine with a pH-value of less than six [6].

Discussion

Urinary TXB₂ was found to be an early and sensitive indicator of renal allograft rejection. The major source of immunoreactive TXB₂ is considered to be intrarenal by cells involved in immunological events, especially platelets and macro-
<table>
<thead>
<tr>
<th>Time (days)</th>
<th>-6</th>
<th>-4</th>
<th>-2</th>
<th>0(^1)</th>
<th>+2</th>
<th>+4</th>
<th>+6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume</td>
<td>1.7 ± 0.3</td>
<td>1.3 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.2(^*)</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>(1/24hr)</td>
<td>(1.1 - 4.0)</td>
<td>(0.1 - 2.3)</td>
<td>(0.1 - 1.2)</td>
<td>(0.1 - 1.8)</td>
<td>(0.1 - 2.0)</td>
<td>(0.1 - 2.4)</td>
<td>(0.1 - 3.3)</td>
</tr>
<tr>
<td>Cl(_C)</td>
<td>26±6</td>
<td>24±5</td>
<td>27±7</td>
<td>21±5</td>
<td>25±8</td>
<td>28±7</td>
<td>28±6</td>
</tr>
<tr>
<td>(ml/min/1.74m(^2))</td>
<td>(5–38)</td>
<td>(5–52)</td>
<td>(5–52)</td>
<td>(5–49)</td>
<td>(5–64)</td>
<td>(10–61)</td>
<td>(5–56)</td>
</tr>
<tr>
<td>TXB(_2)</td>
<td>0.36±0.08</td>
<td>0.34±0.06</td>
<td>0.41±0.05</td>
<td>0.79±0.09(^*)</td>
<td>0.64±0.10</td>
<td>0.69±0.12</td>
<td>0.30±0.03</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>(0.18–0.58)</td>
<td>(0.18–0.52)</td>
<td>(0.22–0.80)</td>
<td>(0.19–2.16)</td>
<td>(0.26–2.33)</td>
<td>(0.19–2.50)</td>
<td>(0.20–0.52)</td>
</tr>
<tr>
<td>(\beta)MG</td>
<td>8.2±2.1</td>
<td>6.4±1.8</td>
<td>6.2±2.4</td>
<td>7.4±2.3</td>
<td>12.8±3.6</td>
<td>14.2±3.4</td>
<td>10.8±2.8</td>
</tr>
<tr>
<td>(µg/ml)</td>
<td>(3.2–11.3)</td>
<td>(3.1–10.7)</td>
<td>(2.8–10.2)</td>
<td>(3.4–12.0)</td>
<td>(2.7–19.7)</td>
<td>(4.6–18.8)</td>
<td>(3.8–16.4)</td>
</tr>
</tbody>
</table>

\(^1\) Day of clinical diagnosis of allograft rejection, −/+: Days before/after clinical diagnosis of rejection, Cl\(_C\): Endogenous creatinine clearance; mean ± SEM (range), n = 24; \(^*\): p<0.05, in comparison to day six before clinical diagnosis of rejection.
phages which are known to accumulate in the allograft during rejection. An extrarenal origin of TXB₂ is described only for venous thromboembolic disease [2,7]. No such events could be observed in our patients. Renal TXB₂ excretion is not responsive to vasoactive hormones contrary to PGE₂ [8], whereas fluid restriction seems to influence TXB₂ excretion by passive reabsorption of TXB₂, associated with ADH-induced water reabsorption [8]. This mechanism is of minor importance in kidney transplant patients who are kept in a state of hypervolemia during the initial phase after transplantation. During effective rejection therapy the urinary TXB₂ excretion returned to basal values indicating no further activation of immunocompetent cells and platelets. The spontaneous decrease of elevated urinary TXB₂ levels in five cases without conspicuous clinical symptoms of rejection implicates the possibility of a spontaneous suppression of slight rejection episodes by continuation of the standard immunosuppressive therapy. In all these cases retrospective analysis of the clinical data revealed the existence of disturbed allograft function or increased body temperature as indicators of an immunological event.

No specific effect of immunosuppressive therapy with cyclosporine, azathioprine plus steroids or additional rejection therapy with high dose steroids could be observed on the excretion of TXB₂ and βMG. This is surprising as steroids possess the capacity to suppress the activity of phospholipases and by this mechanism inhibit the release of membrane-bound phospholipids which are precursors of the arachidonic acid cascade.

No elevated TXB₂ excretion was observed during urinary tract infection, acute tubular necrosis, and generalised infections whereas urinary βMG was increased during these events in addition to episodes of renal allograft rejection. This can be explained by the complex mechanism of renal βMG excretion which depends on serum βMG concentration, glomerular filtration rate and proximal tubular cell function and on increased generation of βMG during inflammatory diseases [9,10]. The present data indicate that the determination of urinary TXB₂ permits a non-invasive evolution of renal transplant rejection. Because of its higher specificity and chemical stability TXB₂ is a more reliable indicator of renal allograft rejection than βMG.

References

5. Steinhauser HB, Lubrich I, Schollmeyer P. Clin Hemorheol 1983; 3: 1