PART VII
IMMUNOLOGY—REJECTION
Chairman: Dr M Legrain
Urinary Excretion of NAG and FDP in Acute Renal Graft Rejection

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Summary

Determination of the urinary excretion of N-acetyl-D-glucosaminidase (NAG) and fibrin degradation products (FDP) made it possible to make a diagnosis of 25 out of 26 acute rejection episodes at least 24 hr before deterioration in renal function occurred.

Of the two tests, the estimation of daily urinary NAG is the most practical for routine clinical use. This test alone permitted early diagnosis in 21 out of 26 episodes.

In 9 out of 11 episodes in which both estimations showed an increase, the rise in FDP occurred before the rise in NAG. This is consistent with the view that in some forms of rejection, at least, intravascular fibrin deposition occurs first and causes ischaemic damage to renal tubular cells and consequent deterioration in function.

Introduction

The early diagnosis of acute renal graft rejection is an important factor in the management of the patient who has received a renal transplant. It ensures immediate augmentation of immunosuppressive therapy, thus preventing or reversing deterioration in allograft function. Regular monitoring of the urinary lysosomal enzyme N-acetyl-D-glucosaminidase (NAG) has been found by our unit to be useful in making the early diagnosis of rejection (Wellwood et al, 1973; Sandeman et al, 1973). Other groups have documented the value of determinations of urinary fibrin degradation products (FDP) for this purpose (Clarkson et al, 1970; Naish et al, 1973).

In the present study these two parameters were compared in order:

(i) To assess their relative accuracy and usefulness for the early diagnosis of rejection, and
(ii) to study the pathogenesis of the acute rejection episode.
PATIENTS AND METHODS

Urine Samples

Studies were undertaken of 882 urine samples from 54 patients with renal transplants. Of these 54 patients, 21 were recently transplanted (16 cadaver and 5 living related donors) and daily determinations of urinary NAG and FDP were carried out on the morning urines from these patients from the day of transplantation until discharge from hospital. The remaining 33 patients had been transplanted at least 3 months before the study began and between 3 and 8 determinations were carried out on urine obtained from these patients at the outpatients visits. In addition, 48 random urine specimens were obtained from 48 healthy control subjects. All urine samples were collected without preservative and were either estimated immediately or stored at \(-20^\circ\text{C}\).

Chemical Methods

NAG was estimated by routine fluorimetric enzyme method using a synthetic substrate (Leaback and Walker, 1961). By using an Autoanalyser method it is possible to obtain 30 results per hour on unconcentrated urines (Tucker, in preparation). The results are expressed in nmol/hr per mg of urinary creatinine. For the FDP assay, urine was concentrated by overnight dehydration in polyethylene glycol. To remove contaminating fibrinogen, thrombin was added to the urines and the specimens absorbed with preserved sheep red blood-cells (Clarkson et al, 1970). We use the tanned-red-cell haemaglutination immunoassay technique (TRCHII) as originally described (Merskey et al, 1966) to determine the FDP in the concentrated absorbed urine. The results are expressed in microgrammes per millilitre of urine.

Diagnosis of Rejection

For the purposes of the analysis of the results obtained in this study, an acute rejection episode has been diagnosed only when each of the following criteria was met:

(i) The clinician in charge of the case made an independent diagnosis of rejection on the basis of constitutional signs and renal functional assessment at the time.

(ii) The episode was accompanied by a definite deterioration in the biochemical evaluation of renal function in the absence of bacteriological evidence of urinary infection.
RESULTS

Controls

The urine specimens obtained from the healthy control subjects contained 47 (SD ± 17) nmolNAG/hr/mg urinary creatinine. No detectable FDP was found in 36 and a maximum concentration of 0.25 μg FDP/ml urine in 12 of these control subjects.

Patients with Stable Transplant Function

Estimations in 29 patients with renal allografts in whom function was considered stable by all clinical and biochemical criteria for 3 months or more, showed a mean NAG value of 144 (SD ± 62) nmolNAG/hr/mg urinary creatinine. The mean urinary output of FDP was 0.28 (SD ± 0.31) μg/ml urine. There was no correlation (p>0.05) between NAG and FDP elimination in the urines of the 29 patients with stable renal function.

Patients with Haematuria (Figure 1)

When haematuria occurs, usually as a result of post-transplant surgical complications, measurements of FDP are unreliable. Nevertheless, NAG excretion remains unaltered in the absence of rejection. It is interesting to note that two days after the haematuria episode the FDP returns to a stable range.

![Figure 1. Urinary NAG and FDP during 10 episodes of haematuria due to surgical complications following renal grafting. Each point is the mean ± SEM.](image-url)
Patients on Gentamicin (Figure 2)

Measurements of NAG in patients receiving the nephrotoxic antibiotic gentamicin are unreliable for the diagnosis of rejection. The mean values of NAG urinary excretion found during rejection were 1080 nmol/hr/mg urinary creatinine (SD ± 525), and as shown in Figure 2 after two days of gentamicin the values found were higher than this. Nevertheless FDP excretion remains stable in the absence of rejection. The mean excretion of FDP after 10 days of gentamicin is, in fact, slightly lower than on the first day. This is probably due to the effect of gentamicin on the urinary infection.

![Figure 2. Urinary NAG and FDP during 11 courses of gentamicin in renal graft recipients. Each point is the mean (± SEM).](image)

Patients with Acute Rejection Episodes (Table I)

Acute rejection episodes were diagnosed on 26 occasions according to the criteria given above. In 25 of these episodes (96%) an increase in one or other or both of the two parameters gave at least 24 hr warning of rejection before a diagnosis based on a change in renal function. In 16 of these episodes there was a rise in both NAG and FDP. Of the remainder, there were five episodes which occurred in the immediate post-operative period and in which the presence of haematuria invalidated the FDP determinations. Nevertheless, the estimation of NAG remained a reliable index of rejection on these five occasions. In two episodes, the administration of gentamicin invalidated the NAG results. One of these patients also had haematuria, but in the other a significant early rise in FDP was detected. There
TABLE I. Acute Rejection Episodes (26) arranged to show Numbers with Diagnostic Increases in NAG and FDP

<table>
<thead>
<tr>
<th>NAG</th>
<th>FDP</th>
<th>Rejections</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>16</td>
</tr>
<tr>
<td>+</td>
<td>Haematuria</td>
<td>5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Haematuria</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26</td>
</tr>
</tbody>
</table>

NAG measurements useful in 21 (81%)
FDP measurements useful in 20 (77%)
NAG + FDP measurements useful in 25 (96%)

were three episodes out of 24 in patients not receiving gentamicin in which no significant rise in NAG excretion was found before the clinical diagnosis. In these three episodes a warning rise in FDP occurred. A rise in FDP was detected before biochemical deterioration in all the rejection episodes except those six in which haematuria precluded its usefulness. The 16 acute rejection episodes in which there was a rise in both NAG and FDP were analysed. Values of NAG and FDP measured on the days after the deterioration in renal function were significantly correlated (p < 0.01, Figure 3). In 11 out of the 16 acute rejections associated with an increase in both parameters, daily measurements were available for at least five days before the clinical diagnosis. A diagnostic rise in FDP gave four days warning of rejection in 8 patients and the NAG gave three days warning in six patients (Figure 4). The elevation in FDP preceded the rise in NAG in nine patients out of 11. In the remaining two, elevations in both parameters occurred on the same day; in the first patient four days before the clinical diagnosis and in the second 24 hr before. In no case did the rise in NAG occur before the rise in FDP. A typical acute rejection episode and the effect of treatment is shown in Figure 5.

DISCUSSION

This comparison of the urinary excretion of NAG and FDP in renal-transplant patients confirms that both parameters are useful early tests for the diagnosis of acute rejection. However, the estimation of FDP is laborious and time consuming and because of this, we recommend the estimation of NAG for routine clinical purposes. This test does not require concentration of the urine, is now fully automated and gives accurate results which are available within a few hours of the morning urine sample being taken. Moreover, the NAG remains a reliable
index of rejection in clinical situations in which the FDP gives false positive results. These are, first, the presence of haematuria in which Braun and Merrill (1968) suggested that urokinase activity in the urine might be responsible for the formation of FDP from fibrinogen, and second, urinary infection (Whitworth et al, 1973). Both of these situations are common in the critical early-post-transplant period when speedy diagnosis is most important.

Nevertheless, in certain situations there is an advantage in having both tests available. FDP estimations are reliable in the diagnosis of rejection when the patient is receiving gentamicin which raises the urinary NAG output (Wellwood et al, 1974) and, possibly, other nephrotoxic drugs. We have found three rejection episodes in which the rise in NAG did not precede the clinical diagnosis but which the FDP gave reliable early warning. In one of these cases our criteria for the diagnosis of rejection were reinforced by histological findings. The frequency with which this sequence may be encountered is uncertain.
Figure 4. Timing of diagnostic rise in NAG and FDP in 11 acute rejection episodes associated with a rise in both parameters.

Figure 5. NAG and FDP values during an acute rejection episode and its treatment.

The present results are of interest in relation to the pathogenesis of acute rejection episodes. NAG is a lysosomal enzyme of the renal tubular cells (Price
and Dance, 1967; Taylor et al, 1971). Plasma NAG levels do not rise with urinary increases during rejection episodes (Wellwood et al, 1973). This suggests that the rise in urinary NAG excretion which accompanies rejection is due to damage to tubular cells. This has been corroborated by unpublished isoenzyme studies (Tucker, in preparation).

Immunofluorescent studies of biopsies from renal transplants have shown fibrin deposition in the peritubular capillaries during rejection episodes (Busch et al, 1971). Our present findings that the rise in urinary FDP occurred before the rise in urinary NAG in 9 out of 11 acute rejection episodes and that in no episode did the rise in NAG precede the rise in FDP suggest that deposition of fibrin in the renal vasculature precedes tubular cell damage. This is consistent with a view that ischaemia is the link in the pathogenesis of structural damage and functional deterioration of the renal graft (Kountz et al, 1963).

Acknowledgement

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Open Discussion

GONICK There are certain problems in using urinary enzymes as a diagnostic tool. First, for most enzymes both inhibitors and activators are present in the urine in variable quantities. These compounds must be removed by dialysis. Did you do this? Although we spent something like three or four years in collecting daily urines to measure the urinary enzyme activity, we finally gave up the technique because simple observations such as change in urine volume, increases in blood pressure or a small increase in serum creatinine were almost always sufficient to give us the critical information.

GARCIA As far as I know it is not necessary to dialyse urine for NAG. FDPs are probably better measured in urine than in plasma.

GONICK As a matter of simplicity it certainly is easier to work with urine, but one is restricted to using plasma in the oliguric phase of acute tubular necrosis in the immediate post-transplant period.

WIBELL (Sweden) Am I right in supposing that this enzyme, like lysozyme, αβ2 microglobulin, leaks into the urine when you have reduced GFR, and in that case at about what serum creatinine level have you come down to?

GARCIA NAG comes from the tubular cells and is not filtered.

WIBELL What is the molecular size?

GARCIA It’s about 160,000.

F P BRUNNER (Switzerland) Could you tell us whether you now determine NAG and FDP in the urine routinely after each transplant and whether you decide from these measurements whether you are going to treat with methyl prednisolone in 1g injections or not?

GARCIA Yes, we collect urine from each transplant patient every day and measure NAG and FDP. We consider an increase in NAG of about 25% of the preceding day a sign of rejection. An increase of 100% in FDP is also taken as indicating rejection.

BRUNNER Do you start treatment with 25% increase, no fever, and normal renal function?

GARCIA Yes. Our experience suggests that waiting is not justifiable.

CHAIRMAN But Dr Garcia how do you rule out false positive tests?

GARCIA At first we did not treat patients until the creatinine rose.

CHAIRMAN And you never had false positives?

GARCIA We had three false negatives with NAG.