MINIMUM STEROID REQUIREMENTS IN RENAL TRANSPLANT PATIENTS MONITORED BY URINARY FIBRIN DEGRADATION PRODUCTS AND COMPLEMENT

J L Anderton, L Fananapazir, Maureen Eccleston

Western General Hospital, Edinburgh, United Kingdom

Summary

The purpose of this study was to define the minimum steroid requirement in patients with a well established renal transplant, monitoring rejection by urinary fibrin degradation products (FDP) and complement (C₃) measurements.

Urinary FDP and C₃ were measured daily over two years in ten patients who had a renal cadaveric transplant. Steroid therapy was reduced step-wise over an average period of fifty weeks to minimum values (range 5–10 mg, mean 7.0 mg prednisone). Three patients developed rejection when taking 7.5 mg prednisone for 10, 21 and 50 weeks respectively. In these three patients urinary FDP excretion rose markedly 12, 10 and 8 weeks respectively prior to the diagnosis of rejection and had fallen to pre-rejection values by the time any significant changes were observed in renal function. C₃ appeared in the urine of two of the three patients who had graft rejection, heralding the diagnosis by 14 and 11 days respectively.

The minimal steroid dosage varied from 0.06 to 0.24 mg prednisone/kg body weight (mean 0.11) and the three patients who rejected did so on doses of 0.10, 0.13 and 0.16 mg/kg. Doses of prednisone less than 10 mg per day risk the induction of rejection, depending upon the individual response of the patient.

Introduction

Renal transplantation is now an established form of treatment for chronic renal failure. However, rejection still accounts for considerable loss of transplanted kidneys, despite the use of steroids and other forms of immunosuppression. Maintenance dosages of steroids in patients with good renal function are frequently associated with increased morbidity. Attempts are therefore made to reduce the steroid dosage to minimum levels compatible with graft survival. Patients vary considerably in this respect and minimal steroid dosages are often achieved by trial and error. Attempts have been made to define the
lowest dose below which rejection occurs — the individual threshold dose.1
One of the purposes of this prospective study was to determine the feasibility
of this concept using recently developed diagnostic approaches.

The diagnosis of kidney transplant rejection is unreliable and often incon-
clusive. Detection of rejection is therefore frequently late and as a consequence
irreversible damage to the graft may occur. Urinary FDP and C3 excursions
have been claimed to be useful markers of early renal transplant rejection2-6.
In this study we have tried to determine their value as monitors of late trans-
plant rejection during a period of steroid reduction in patients with stable
renal function.

Patients and Methods

Ten patients who had received a renal transplant 2 to 7 years previously (mean
3 years 7 months) were studied. All had stable renal function at the beginning
of the study and had been free from rejection episodes in the previous six
months. The plasma creatinine was less than 0.10 mmol/L in 9 of the 10
patients and in the remaining patient 0.11—0.15 mmol/L. The blood urea
ranged from 5 to 11 mmol/L (mean 7.0 mmol/L). The initial dose of predni-
sone ranged from 12.5 mg to 20 mg (mean 18.5 mg/day) and Imuran from
100 mg to 225 mg/day (mean 145 mg).

All patients were assessed on a two weekly basis throughout the study for
renal function (blood urea, plasma creatinine, urinary volume, protein excre-
tion, urine microscopy and an MSU culture) and clinical evidence of transplant
rejection (oliguria, fever, renal tenderness and hypertension). Each day through-
out the study they collected an early morning 20 ml urine sample into a uni-
universal container and posted it to the laboratory.

Urine samples were dialysed against tap water, concentrated in polyethylene
glycol at 4°C overnight and stored at -18°C until assayed at weekly intervals
for FDP and C3.

FDP was measured by the tanned red cell haemagglutination inhibition
immunoassay technique7, with certain modifications8. C3 was measured by
the Mancini technique9 and total urinary protein was measured by the biuret
method10. Urine was examined for blood using Labstix.

After a stable period of renal function and urinary FDP excretion had been
observed (mean 6 months), the steroid dosage was reduced by 2.5 mg decre-
ments at approximately two monthly intervals. To prevent precipitating irre-
versible rejection, great caution was exercised in the timing of steroid reduction.
No further steroid reduction was carried out until any slight deterioration in
the biochemical indices had settled. The lowest dose of prednisone achieved
ranged from 5 mg (three patients), 7.5 mg (six patients) to 10 mg (one patient)
(mean 7 mg).

Results

The mean (± SD) urinary FDP excretion in a control group of eight normal
subjects studied daily over a period of one month was 0.10 ± 0.14 mg/L. In
each of nine patients the mean urinary FDP excretion was not significantly different from the control group (range 0.01 ± 0.06 mg/L to 0.11 ± 0.32 mg/L). In one patient it was significantly higher (0.17 ± 0.30; t = 3.93, p = < 0.0005). Most of the values over the two years were within 2 SD of the normal mean, i.e. less than 0.4 mg/L. However, spikes of 0.91 mg/L to 4.6 mg/L were observed in most patients which lasted only a few days. Figure 1 shows

Figure 1. Daily urinary FDP (upper line) and effect of taking the mean of seven daily estimations (middle line) and fourteen daily estimations (lower line) on the graphical representation

a ten week period of FDP excretion in a typical patient not undergoing transplant rejection and the effect on the baseline of recording the means of seven and fourteen consecutive daily samples. In the non-rejecting patients the two weekly mean FDP never rose to values of greater than 0.6 mg/L. In the three patients who developed transplant rejection, urinary FDP rose from basal values at 12, 10 and 8 weeks and reached two weekly mean values of greater than 0.6 mg/L 9, 4 and 3 weeks before the clinical diagnosis of rejection (see Figure 2). Figure 3 shows by comparison the urinary FDP excretion in one patient who was free of rejection throughout the study.

An increase in total urinary protein excretion occurred much later and was associated with deterioration in the other biochemical indices. It was not related to urinary FDP excretion.

C₃ was not found at any time in any of the patients during the non-rejection period. However, C₃ was detected in the urine of two of the patients
Figure 2. The effect of reduction in prednisone dose on plasma creatinine, blood urea, urinary protein, and urinary FDP in a rejecting patient.

Figure 3. The effect of reduction in prednisone dose on plasma creatinine and urinary FDP in a non-rejecting patient.
who rejected, and preceded the diagnosis by 14 and 11 days respectively. The third patient had a more chronic and milder rejection episode with a comparatively smaller rise in urinary FDP excretion.

Discussion

The diagnosis of renal transplant rejection is normally made by observing a fall in urine volume and a rise in blood urea and creatinine. Occasionally fever, renal tenderness and hypertension accompany the rejection process. However, the immunological changes which instigate rejection probably take place some days, weeks or months before there are perceptible changes in renal function. Various techniques have been used to detect these changes but all have numerous drawbacks and have not reached the stage of routine use in clinical practice.

The excretion of urine FDP has been claimed to be a good indicator of early renal transplant rejection and often precedes the biochemical changes by some days. The rejection episodes studied by these workers were acute or subacute and no long term study of urinary FDP excretion in these patients has been carried out to monitor late rejection episodes which occur when conventional steroid dosages are reduced. In the study described above, urinary FDP appeared to be an excellent marker of renal transplant rejection and preceded the biochemical changes by some weeks in all patients. For the purpose of this study, our policy was to treat rejection episodes only in the presence of traditional biochemical evidence. However, the fact that urinary FDP excretion rose before the clinical and biochemical diagnosis of rejection was made suggests that earlier treatment could be instituted which might abort rejection and improve graft viability.

One purpose of the study was to detect the lowest level of prednisone at which renal transplant function could be maintained without rejection. Owens et al. observed no deterioration in renal transplant function in six patients who discontinued all immunosuppressive therapy for an average of 27 months. Turcotte et al. sought to define the minimum dose of steroids for 42 patients, below which rejection was likely to occur — ‘the minimal threshold dose’, and the mean prednisone dose at the time of 75 rejection episodes was 0.27 mg/kg (range 0.08 to 0.6 mg/kg). This high mean value and wide range was probably due to the fact that their results included patients with early as well as late rejection episodes. All their patients with a renal transplantation history of greater than 18 months had rejection episodes when on doses of prednisone less than the above mean. Only five of the rejection episodes occurred more than 24 months post renal transplantation and in these patients the mean ± SD prednisone dose at the time of rejection was 0.13 ± 0.03 mg/kg. In our study, which was carried out at a much later time in the transplantation history (2—7 years), the mean maintenance dose of prednisone was 0.3 mg/kg initially (range 0.16 — 0.42 mg/kg). In seven patients we were able to reduce this further to a mean of 0.11 mg/kg (range 0.06 to 0.24 mg/kg), without precipitating a rejection episode. These low steroid doses would conventionally be regarded as dangerous, in terms of the risk of rejection. In the remaining

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three patients rejection occurred at 0.10, 0.13 and 0.16 mg/kg respectively. As Figure 4 shows, there was no significant difference between the steroid therapy of the two groups and the five late rejection episodes studied by Turcotte et al.

Our experience would suggest that it is difficult to define an 'individual threshold dose'. A reliable index of renal transplant rejection, such as urinary FDP, is extremely useful in this situation to predict potentially irreversible rejection which may occur at these relatively low steroid doses. We would therefore recommend that urinary FDP should be analysed routinely, particularly in patients with stable renal function who are undergoing a period of steroid reduction.

Estimation of urinary FDP on the random sample may be misleading as in
all the patients studied, there were occasional small rises over a few days. The exact aetiology of this pattern of urinary FDP excretion is uncertain. Although urinary tract infection has been implicated as a contributory factor, in our study these spikes were independent of thirteen episodes of urinary tract infection which occurred in half of the patients. Urinary FDP excretion due to the rejection episode was greater and sustained over a longer period of time and could be differentiated from the basal pattern of excretion by studying the means of fourteen consecutive daily samples.

C₃ has been observed in renal biopsies of patients with graft rejection and glomerulonephritis. Hoq et al. reported one case of acute transplant rejection with a rise in urinary C₃ excretion, coincident with biochemical changes, within ten days of transplantation. He showed close correlation between urinary FDP and C₃ excretion in the early transplant period. In our study, concerned with long term transplants, two of the three patients with rejection showed a rise in C₃ excretion preceding the diagnosis by fourteen and eleven days respectively. However, the peak urinary FDP and C₃ excretions were dissociated. It therefore appears that urinary C₃ may also be useful in forewarning impending rejection. As no C₃ was found at any time in association with the non-specific FDP peaks, the combination of the two was found to be particularly useful in the diagnosis of rejection.

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Open Discussion

PARSONS (London) I was very interested to see that you predicted rejection some ten days before it happened, and in one case ten weeks before it happened, by using FDPs in the urine. Can you tell us what is actually happening in the kidney at this time?

ANDERTON We did not carry out biopsy at that stage, so I cannot. One assumes that the immunological mechanisms associated with the rejection are taking place, and that fibrin is being laid down and fibrinolysis is taking place and FDPs are appearing in the urine. This is purely conjecture.

PARSONS But what about the timing?

ANDERTON This does occur, some ten or in one case twelve weeks prior to the clinical and the biochemical evidence of rejection. I think in patients who have such good renal function, the deterioration of renal function will occur because of the immunological mechanisms and this will only be picked up by serum creatinine some weeks or months after the initial process has taken place. You have to lose half of your kidney before your blood urea rises above normal levels. This is the problem in these patients with excellent renal function.

WEGMULLER (Berne) You did not mention anything about the amount of C₃ excretion in the urine. Did you find a correlation between C₃ excretion in the urine and the C₃ level in the plasma?

ANDERTON There was no C₃ in the urine of the patients who had no rejection. In those two patients who showed an elevation some fourteen and ten days before the rejection, the urine C₃ levels were of the order of 10 mg/L. We did not measure plasma C₃ levels.

McGOWAN (Belfast) In these patients in whom you found rejection episodes, how did you then proceed?

ANDERTON We treated rejection episodes with three doses of Solu-Medrone, 1 gram, and put the patient back up to about 25 mg prednisone for two to three weeks and then back to the original starting dose, 15 or 17.5 mg prednisone, and then very gingerly, not included in the study, reduced the dose to certainly no lower than 12.5 mg prednisone.

HANSEN (Aarhus) It is very interesting that you can predict rejection crisis very early and as we have been interested in late rejection, especially glomerular rejection with proteinuria, I want to ask you how long before any sign of proteinuria did your signs of rejection appear?

ANDERTON There was no correlation between the urine FDP excretion and proteinuria. In three patients who rejected, proteinuria appeared about two or three weeks before the serum creatinine was elevated and was not associated with urine FDP excretion.

CZARNIECKI (Warsaw) Did you observe any direct correlation between the
FDP level and the dosage of steroids. As you know from the theoretical point of view, the administration of steroids increases the level of FDP.

ANDERTON We saw no correlation between the doses of steroid and the urinary FDP during the control period in any patients. Following steroid reduction in seven patients who did not reject, the FDP levels remained either absent or at very low levels, less than 0.5 mg/L. There was no relationship between those levels and the steroid dose.

BARNES (Birmingham) I would like to sound a note of caution because in the past we did very similar things to you, looking for FDPs in these long-term patients, and quite frequently found peaks of FDP excretion which were not associated with any rejection episode. We were not specifically looking at reducing the dose of steroids at this time. In our grafts we go down to about 10 mg and stay there. The big question that worries us is, how often should you see these patients? How often should you actually bleed them and what tests should you do? Our own impression is that once they are out beyond the first year or so and they have prednisone doses down to about 10 mg, which is a fairly safe level in most patients, the number of occasions when you need to see them and the number of tests you want to do, become remarkably few. Hearing some of the other papers in this session, how much real value are these elaborate tests to the patients and what is going to be the optimum way of managing these long term patients?

ANDERTON I agree with you that spikes of FDP excretion in the urine should be ignored and we have taken the mean of fourteen samples to give a single individual reading. If this mean of fourteen samples is, however, elevated above 2 mg/L we consider that to be very significant. With regard to your second point, I heartily agree that these patients who have settled down following renal transplant should visit hospital as little as possible. The beauty of this test is that it can be done by patients simply sending urine to the laboratory by post and one does not have to carry out blood tests. We would normally just see these patients at monthly or six weekly intervals and we certainly would not carry out these tests routinely on all patients. I think from the practical point of view if you have a patient who is on 10 mg or so and for one reason or another you want to lower the dose, it is useful to monitor this.