A Simple Test for Early Detection of Severe Renal Homograft Rejection

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Summary

Adding urine to a standard buffered fibrinogen solution and then coagulating it with thrombin gives reproducible coagulation times with normal urines. Coagulation of fibrinogen by thrombin is prolonged in acid solutions with a pH below six. Urines of high acidity lower the buffer pH of the fibrinogen solution below a value of six thus rendering the system uncoagulable or significantly prolonging the coagulation time. With this test system we found that out of 16 severe homograft rejections 15 were accompanied by a high acid excretion in six-hour urine specimens. Ten of these acid episodes became apparent 12 to 48 hr before clinical symptoms and before elevation of the serum creatinine could be detected.

Introduction

Excretion of fibrinogen degradation products (FDP) in the urine of patients with renal allografts is a well known fact (Braun and Merrill, 1968; Zühlke et al, 1969). In experiments to detect these FDP's by their antithrombin property we found an inhibition of fibrin coagulation in our test system. This prolongation of coagulation time nearly always occurred before the clinical signs of a rejection crisis. Because we could not detect FDP's in urine samples by immunological methods (Hulme and Pitcher, 1973), we searched for another explanation for this phenomenon. As is shown below this prolongation of the fibrinogen coagulation time in our test system was caused by the presence of buffers.

METHODS

Sixteen patients who had received cadaveric renal transplants were followed up by testing six-hour urine samples. A total of 27 rejection crises were observed. The compatibility matches of the transplants were D and E, with only one C. At the time of transplantation patients received 1 g of methylprednisolone and 5 mg/kg body weight azathioprine intravenously, the prednisolone dosage being
reduced subsequently from 200 mg on the first post-operative day to 40 mg at
the ninth post-operative day. Azathioprine was given according to the leuco-
cyte count, averaging 1.5 to 2 mg/kg body weight per day.

The urine samples were stabilized against bacterial deterioration by a 10%
solution of thymol in isopropanol. Each six-hour sample was refrigerated im-
mEDIATELY after collection.

Our fibrin coagulation test system which proved applicable to urine consisted
of a bovine fibrinogen solution of pH 7.35. A quantity of 60 mg bovine fibrinogen
(Behring) was added to a solution of 4.5 ml distilled water, 9 ml concentrated
Owren-veronal buffer pH 7.35 and 1.5 ml isotonic citrate solution. Coagulation
was performed with antithrombin reagent (Roche), one vial dissolved in 5 ml
distilled water. Urine (0.1 ml) and fibrinogen solution (0.1 ml) were mixed at
37°C and the coagulation started by adding 0.2 ml of the thrombin solution, the
coagulation time being measured by the method of Schnittger and Gross. In nor-
mal urines coagulation times varied between 9 and 22 sec. Titration of the urine
was performed with a Radiometer autotitration device. We used 3 ml of urine
with 1 ml of distilled water and titrated the urine with 0.1N NaOH up to a pH
of 7.4.

A rejection crisis was accepted as severe when the serum creatinine increased
by 1 mg% during the first four days following the clinical symptoms character-
istic of a rejection episode, namely an enlarged and painful transplant, relative
lymphocytosis, and a fall in urine sodium. On appearance of these clinical symp-
toms 1 g of methylprednisolone was infused.

RESULTS

Figure 1 shows the prolongation of the coagulation time with increasing acidity.
There was a sharp drop in coagulation time between a pH of 5.8 and 5.7. When
we compared the coagulation times of urine samples with the relative acidity
titrated to pH 7.4 we found a correlation coefficient of 0.70 (Figure 2).
(Y = 2.3 + 0.9 \times \text{relative acidity in mEq/l}). From this we concluded that the
prolongation of coagulation time is dependent on the relative acid excretion. We
proved this assumption by neutralizing the urine samples, thus normalizing coag-
ulation time. Figure 3 demonstrates the characteristic acid spikes observed in con-
nection with severe homograft rejection crisis. The coagulation time of the test
system rose to over 30 sec and the relative acidities of the urine portions were
higher than 30 mEq/l (titrating only to pH 7.4); in the preceding or following
six-hour urine samples the relative acidity was more than 25 mEq/l. Usually the
coaulation system is rendered uncoagulable by such strong acid excretion.
During the first days after renal transplantation our patients normally excreted
rather low-acidic urines once the creatinine had dropped below 2 mg%. As long
as large amounts of creatinine are eliminated, the urine titrable acidity is high
because of the low pK value for creatinine pK = 4.97 (Pitts, 1972). The post-
Figure 1. Prolongation of coagulation time with dropping pH.

Figure 2. Correlation of the relative urine acidity (titrated to a pH of 7.4) to the coagulation time of the same urine portion.
operative values ranging between zero and 25 mEq/l, averaging 8 mEq/l. With one exception, all of our observed severe homograft rejection crises occurred during the first 16 days after transplantation. The results of our investigation are compiled in Table I, where it is seen that out of 16 severe homograft rejection crises,

Table I. Number of Acid Spikes and Rejection Episodes Observed

<table>
<thead>
<tr>
<th></th>
<th>Severe Rejection Crisis</th>
<th>Light Rejection Crisis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejections</td>
<td>16</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>Acid spikes</td>
<td>15</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Acid spikes missing</td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Preceding 48 hr</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Preceding 12 to 24 hr</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>At time of clinical symptoms</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Following clinical symptoms</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Acid spikes without rejection</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>
15 were associated with acid spikes in their six-hour urine samples. On only 10 occasions did the acid spikes precede clinical symptoms. In four cases we observed non-specific high-acid excretion; one proved to be an abscess surrounding the transplant, the other a lung infection with fever.

**DISCUSSION**

From the results listed in Table I we conclude that the detection of acid spikes is of no value in diagnosing mild rejection not followed by a rise in serum creatinine. The two infective states accompanied by high acid excretion indicate that the acid spikes observed are probably non-specific. Because the risks of acquiring an infection increase with time after transplantation, the high relative acid output is only reliable for detection of severe homograft rejection in the first 14 days after transplantation, when most severe homograft rejections take place. As long as the clinical symptoms of rejection do not subside, the relative high acid excretion continues, although the absolute acid values vary too much to allow an interpretation of trends. As Figure 3 shows, acid output is only impaired when the serum creatinine reaches 4 to 6 mg%. The coagulation test is not simply another method for determination of urine titrable acidity, this is

![Figure 4. Titration curves of acidic urine portions and of a 0.15 m primary sodium phosphate solution.](image-url)
obvious from the relatively low correlation coefficient and from the fact that it was not possible to depress the pH of the fibrinogen solution below six with stoichiometric amounts of primary sodium-phosphate corresponding to the titrated acidity of the urine portions. This is probably explained by the relatively high pK of the primary sodium-phosphate of 6.8. The shape of the titration curves of urine portions compared to a solution of primary sodium-phosphate in Figure 4 is in agreement with the hypothesis that organic acids with low pK value—as for instance β-hydroxybutyric acid, which is the main buffering substance in diabetic acidotic urine (Pitts, 1972)—are primarily responsible for the effect observed with our test system.

References

Braun, W E and Merrill, J P (1968) New England Journal of Medicine, 278, 1366
Hulme, B and Pitcher, P M (1973) Lancet, i, 6
Pitts, R F (1972) Physiologie der Niere, Schattauer Verlag, Stuttgart

Open discussion

GONICK I must confess that I am rather confused. Dr Better of Israel, who is in this audience, has shown quite clearly that in patients with rejection there is a relatively high urine pH following an acid stimulus. Many investigators have also shown that with rejection, hyperchloraemic acidosis is quite common and this disappears as the rejection is terminated. A common problem in discussing titratable acid excretion is a failure to distinguish between the change in urine pH and the change in phosphate excretion which are the two components of urinary titratable acid. Almost all patients who are placed on immunosuppressive programmes are also placed on antacids because of the concomitant steroid administration. With antacids phosphate is bound in the GI tract and urinary excretion of phosphate is decreased. Another factor which I am sure is important is the secondary hyperparathyroidism which is always present for at least the first few weeks or months after transplantation and which causes a disproportionately high urinary phosphate. Since these factors controlling phosphate excretion in the immediate post-transplant period are poorly controlled, unless phosphate and pH changes are examined separately, any conjectures that you make about titratable acid as an index of rejection would be completely erroneous. The high titratable acid excretion that you found in some patients could be explicable on the basis of a high catabolic state (such as infection) which would be anticipated to cause a high urinary phosphate. My confusion, therefore, is why you focused on titratable acid rather than systemic acidosis or urinary pH which might be a much more useful index of rejection.
OPPOLZER In our experience the interpretation of our results, also the interpretation of enzyme determinations in the urine, depends on the exact collecting of urine over short periods. It makes no sense to make any determination in 24-hr collections where you will never get an acid peak. Secondly, with regard to resorption of phosphate in the gut, all our transplant patients had a vagotomy and pyloroplasty and had taken no antacids. I have to agree with your objection related to secondary hyperparathyroidism because all our patients in the first three to four months after transplantation have a very low serum phosphorus. We measure urine phosphate only twice a week, and they have all rather low TRP so that means that they are excreting high amounts of phosphorus in the urine.

GONICK I am asking why you chose that particular parameter of acid excretion which is the least reliable and gives the least information in contrast to urine pH or ammonia excretion?

OPPOLZER Because it was a very interesting finding and we only found information in the literature that acid output drops during rejection crisis.

BARNES (UK) This is not really on your paper. I want to ask you about the statement that you made that all your patients had vagotomy and pyloroplasty prior to transplantation. How many patients have you done this on and secondly have you had any complications related to the gastrointestinal tract in this group of patients? Has it completely abolished the problem of peptic ulceration?

OPPOLZER To the first question we have done vagotomy and pyloroplasty routinely in about 80 patients and in these 80 patients we had only one bleeding complication — not from an ulcer but from the pyloroplasty.

LEGRAIN I don’t see why because you decreased the time of collection you avoid problems with diet, catabolic rate, infection and so on.

OPPOLZER You are correct, but the acidity and enzyme output varies very much and we found out that the results are easier to obtain by taking very short urine collections.

LEGRAIN Do you have an explanation for this sharp increase in hydrogen excretion?

OPPOLZER No, I haven’t.