Urinary Adenylate Kinase Activity as a Predictor of Renal Allograft Crises

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We examined data on adenylate kinase (EC 2.7.4.3) activity and other clinical chemical values from patients with renal transplants by analysis of variance and discriminant analysis, using various combinations of variables in an attempt to find a predictor of transplant rejection. Some typical data are presented. We conclude that the combination of urinary adenylate kinase and creatinine clearance is the best predictor for identifying patients with transient or destructive renal transplant crises.

Additional Keyphrases: renal transplant · enzyme activity · urine · statistical treatment

The use of urinary enzyme analyses for the early detection or prediction of renal transplant rejections has been reviewed (1). Detection of various kidney diseases by measurements of urinary enzymes has been studied (1–5), but their use as an indicator of renal transplant rejection has not been studied extensively. Data on certain urinary enzymes reportedly may be used to predict renal transplant rejections (6), although some (7) have concluded otherwise. We conclude from a previous study (8) and the present one that the presence of above-normal adenylate kinase (AK; ATP-AMP phosphotransferase, EC 2.7.4.3) activity in urine is a predictor of renal transplant rejections when combined with values for creatinine clearance.

In a previously tested preliminary model of renal transplant crises (8), AK activity in urine increased early, persisted in association with the crisis, and declined with improvement. In that model, we analyzed values for urinary AK and creatinine clearance on various postoperative days by linear discriminant function analysis and suggested that together they were good indexes to renal transplant success during the first six postoperative days. In this study, we have modeled a predictor based on a larger set of clinical values, on analyses of variance, and on discriminant analyses of urinary AK values. We describe here some typical patterns of response to the renal transplant, and we suggest that combining values for urinary AK and creatinine clearance can identify patients with transient or persistent renal transplant rejection crises any time postoperatively.

Materials and Methods

Urine specimens. We stored 24-h urine specimens, obtained postoperatively from 17 renal-transplant patients, at 4 °C for three to four days with negligible loss of AK activity. We showed previously (8) that variations in urine concentration do not significantly affect the ability to detect an abnormal increase in urinary AK. The normal range for urinary AK activity is 0–5 U/L, and urine constituents do not inhibit AK activity (9).

Serum specimens. We obtained serum specimens from patients with impending renal-transplant rejection, as assessed primarily on the basis of oliguria, decreasing creatinine clearance, and increasing urinary AK activity.

Other data. Other clinical laboratory results considered in the evaluation of patients' analyses for comparisons with urinary AK activity included values for electrolytes in urine and serum, serum urea nitrogen, lactate dehydrogenase (EC 1.1.1.27), aspartate aminotransaminase (EC 2.6.1.1), alanine aminotransaminase (EC 2.6.1.2), and results of microbiological assays.

AK assay. We measured AK activity essentially according to Adam (10), as detailed elsewhere (11). The constituents and their final concentrations, per liter, in the assay mixture with the sample were: potassium phosphate buffer (pH 7.0) 20 mmol, phosphoenolpyruvate 0.3 mmol, NADH 0.4 mmol, ATP 8.0 mmol, AMP 8.0 mmol, MgCl2 20 mmol. Sufficient pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase were present so that the coupling system was not rate limiting. The reaction was started by adding the MgCl2 after adding a 100-μL sample to the assay mixture. Activity and reaction rates were determined by measuring the decrease in absorbance of NADH at 340 nm, at 25 °C. The molar absorptivity of NADH, 6.22 × 105 L mol⁻¹ cm⁻¹, was used to convert absorbance to macromoles of product formed. Under these conditions 1 U of activity is that required for the formation of 1 mmol of ADP per minute.

Serial triple renal studies. Patients were evaluated for rejection or success in the therapy by performing serial triple renal studies (12), consisting of angiographic studies with 99mTcPAC compounds to make visible the renal circulation and perfusion and with of 131Iiodohippurate to delineate excretory function.

Statistical analyses. In this study we used the two-way analysis of variance and linear discriminant function programs of the Statistical Package for Social Sciences (13) to test the validity of the chosen predictors after elimination, by graphical analysis, of several common clinical measures as unsuitable.

Results

An uncomplicated, successful renal transplant typically shows the following pattern for urinary AK and creatinine clearance. Urinary AK is increased, sometimes markedly, for the first two postoperative days, then declines to no urinary AK activity or to normal values by the fourth day, in general agreement with observations of other urinary enzyme studies (1, 6, 13). The normal reference interval for urinary AK activity is 0–5 U/L of urine (6). Creatinine clearance may be normal or subnormal initially and remains normal or increases to normal in about a week. Serum creatinine alone is a poor predictor, because normal values are sometimes observed during renal rejection crises.

Figure 1 shows a characteristic pattern for urinary AK, creatinine clearance, and serum creatinine during a patient's recovery from a mild rejection crisis. This patient had a prompt output of urine, but received hydralazine hydrochloride for diastolic hypertension during the first two postoperative days. During the first three postoperative
days, the very high urinary AK activity persisted, the creatinine clearance was decreased, and the serum creatinine increased. On the second and third postoperative day, the patient was given 150 μg of actinomycin-B and 500 mg of methylprednisolone sodium succinate for rejection crisis, and repeated doses of x-ray irradiation on postoperative days 3 through 5. In this instance, the urinary AK remained increased on the third and fourth postoperative days, declining more slowly than is typical after a successful renal transplant. After the third postoperative day, the urinary AK activity declined rapidly, reaching a normal value by two weeks, at which time creatinine clearance had increased to normal and the serum creatinine concentration was normal. The patient was discharged with a functioning renal allograft.

Figure 2 shows the urinary AK pattern of a patient who had a prolonged postoperative period of anuria, owing to acute tubular necrosis that required hemodialysis through the 18th postoperative day. This patient excreted 2.5 L of urine per day by the 20th postoperative day, but the serum creatinine was 61 mg/L, which was interpreted as an early rejection of the cadaver renal transplant. When renal isotope scans on the 21st postoperative day showed a time to peak height of 6 to 8 min and a half-height for peaks of more than 20 min, indicating kidney dysfunction, the patient was treated with 1.5 J/kg (150 rads) of 60Co radiation and intravenous actinomycin. Figure 2 shows the decline in urinary AK activity and increasing creatinine clearance after the 28th postoperative day. The serum creatinine concentration decreased to 25 mg/L by the 28th postoperative day; four days later, renal isotope scans showed a normal (3 min) time to attain maximum peak height. On the 34th postoperative day the patient was discharged. The transplanted kidney has functioned satisfactorily for more than a year.

Figure 3 shows the pattern of these analytes in the postoperative course of a patient who had a hyperimmune acute rejection crisis. There was a persistently high urinary AK activity and declining creatinine clearance, with the serum creatinine concentration increasing rapidly. The urinary AK activity (740 U/L) and the creatinine clearance (0.54 mL/min) reflect extensive tissue destruction and no functioning of the allograft kidney, which was removed on the sixth postoperative day.

Figure 4 shows the urinary AK activity pattern from a patient who had a successful immediate post-transplant period. There was a large volume of urine output, normal serum creatinine values, and normal urinary AK activity by the third day. At the end of the first week, a rejection crisis was suspected because of decreasing urine output, no increasing creatinine clearance values, and gradually increasing serum creatinine values. The serum creatinine values were within the normal range up to the ninth postoperative day. On the eighth postoperative day, the urinary AK activity increased suddenly from normal values. At that time, treatment for a rejection crisis was begun. On the 10th postoperative day, urine AK activity declined to normal values. Subsequent urine samples were not examined for AK activity. The creatinine clearance and serum creatinine values stabilized to normal and the patient was released on the 18th postoperative day with no further complications.

Figures 1–4 show some typical urinary AK activities and creatinine clearance values from the 17 patients we examined who had received transplanted kidneys. All abnormal urine sample values were classified into two groups. Those in Group I had urinary AK activity ≥20 U/L and a creatinine clearance value of ≤30 mL/min at least once. Group II represented all remaining urinary AK activities and creatinine clearance values.

For Group I (n = 28), the mean urinary AK activity was 131 U/L, the mean creatinine clearance was 16 mL/min, and the statistical mean Z was 0.063. All patients who developed
Group II (n = 49), the mean urinary AK activity was 16.0 U/L, the mean creatinine clearance was 48.4 ml/min, and the statistical mean Z was -0.004. All patients who developed transient rejection crises reversed by treatment were in Group II. Any individual could have had sets of urinary AK activity and creatinine clearance values that overlapped in both groups.

First, we performed a discriminant analysis of the data to determine which select variables and input variables (see below) presented the best separation of patients into successful and unsuccessful renal transplant outcomes. Afterwards, two-way analyses of variance was used to determine if the select variable classification of Group I and Group II based on urinary AK activities and creatinine clearance values was the best grouping; whether selection by a patient classification was also represented in Group I and Group II; and what effect different input variables had on the statistical significance of our select variable classifications. The select variables classifications tested were: postoperative day; Group I and Group II (Gp), defined by the limiting restrictions given; and patient (Pt), defined as being or not being one of seven patients who had a renal transplant deteriorating progressively. When introduced as a variable, the grouping by postoperative day was a poor prognostic predictor of renal-transplant success for periods longer than six days (8), and tended to randomize rather than improve discrimination. The Pt classification was less discriminatory than Gp because patients who had a renal transplant deteriorating progressively also had urine values classified in the better prognostic group on some days. The Pt classification was retained as a factor in further testing because the Pt and Gp classifications showed some statistical interaction.

Table 1 summarizes the analysis of variance and the discriminant function analysis of select variables Gp and GpPt with the input variables that yielded the best discrimination and statistical separation of the patients into groups of successful or unsuccessful transplant recipients. The high F values determined by analysis of variance of the data in situations 1 through 3 show that the urinary AK activity and the creatinine clearance values yield a good statistical separation of successful from unsuccessful renal-transplant outcomes. Situation 4 shows no significant difference between the Gp and Pt classifications. The high F and D² values of the mean values of the urinary analytes, determined by discriminant function analysis, also show a very good reliability and statistical separation of Group I and Group II. Situations 5 and 6 indicate that the addition of Pt

Table 1. Summary of Analyses of Variance and Discriminant Function Analyses

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*Degrees of freedom = 1, 73; p < 0.0005 except situation where p = 0.00429. AK activity >90 U/L, creatinine clearance >30 mg/L, and transient not successful = Group I; all others = Group II. a Degrees of freedom = 2, 74; p < 0.0005. *Log urinary AK activity is a better discriminator than urinary AK activity, CrCl, creatinine clearance, D², Mahalanobis distance.

Fig. 3. A hyperimmune rejection crisis

Fig. 4. An early successful renal transplant followed by a rejection crisis and recovery

persistent rejection crises, some of whom lost their transplants, were in Group I; thus, values in Group I were classified as representing destructive transplant crisis. In
restriction to the Gp classification (GpP) enhances slightly the separation of the successful from the unsuccessful renal transplants that is based on urinary AK activity and creatinine clearance values.

Discussion

In our previous study of seven patients with kidney transplants (8), we suggested a significant relationship among the urinary AK activity, the time of increased activity, the severity of the damage, and the type of rejection process during the crises. In this study, using a larger sample size and more variables, we confirmed a pattern of an early postoperative increase in urinary AK activity that declines with improvement or persists in association with a progressing renal-transplant rejection. We determined which variables significantly affected the statistical separation of the successful renal transplants (Group II) from the unsuccessful (Group I). In order of increasing significance, these variables were: creatinine clearance, urinary AK activity, log urinary AK activity, and the combined urinary AK activity and creatinine clearance values. In our proposed model, Group I and Group II are different patient populations with a separation of significance at p < 0.00005, which suggests a small frequency of misclassification. The means of the coordinates for Group I are a urinary AK activity of 131 U/L and a creatinine clearance value of 16.0 mL/min. For Group II, the means are a urinary AK activity of 16.0 U/L and a creatinine clearance value of 48.4 mL/min. Although the values for urinary AK activity and creatinine clearance are abnormal in both groups, we propose that the values of Group I indicate a persistent or destructive kidney-transplant crisis. Increased urinary AK activity, although associated with renal-transplant crises, apparently does not indicate a destructive crisis unless the increase is very high and there is a marked reduction in creatinine clearance. Similarly, others have shown (14) that urinary lactate dehydrogenase is increased in association with renal-allograft crises, but does not indicate a persistent crisis unless the M-type LD isoenzyme predominates.

Our analysis of the proposed model suggests that the combined use of urinary AK activity and creatinine clearance can signal the early onset of renal-allograft crises and serve to monitor the efficacy of treatment. Further study is required to determine whether significant relationships among urinary AK activity, creatinine clearance, diminished kidney function, and other urinary enzymes can better define the patients with successful renal transplants.

This work was supported in part by MBRS Grant RR 08135 from NIH.

References