Urinary Enzyme Excretion by Renal-Transplant Recipients in Relation to Interval after Transplantation

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We determined the urinary excretion of the enzymes aminopeptidase (EC 3.4.11.2), alkaline phosphatase (EC 3.1.3.1), γ-glutamyltransferase (EC 2.3.2.2), and N-acetyl-β-glucosaminidase (EC 3.2.1.30) in two groups of renal-transplant recipients at different times after transplantation (1.6 months and 52 months, respectively). Both groups of patients showed a higher rate of enzyme excretion than did a reference group of healthy persons. More aminopeptidase and N-acetyl-β-glucosaminidase were excreted during the early period after transplantation than later. The time-dependence of urinary enzyme excretion was confirmed in six renal-transplant recipients studied during the course of 15 months after transplantation. There was a general correlation between the extent of urinary enzyme excretion and both the time after transplantation and the daily dose of prednisolone. Therefore, it is necessary to take into account this influence on the extent of urinary enzyme excretion in renal-transplant recipients if urinary enzyme excretion is used as an indicator of renal disorder and especially as an early predictor of transplant rejection.

Additional Keyphrases: effect of prednisolone • monitoring for transplant rejection

Determination of urinary enzyme excretion has proved to be an early predictor of transplant rejection in renal-transplant recipients immediately after transplantation (reviewed in 1), but data on the diagnostic reliability of later determinations of urinary enzyme excretion as a rejection criterion in these patients are few and contradictory (2-4). The reason for this, among other things, is that insufficient data of this kind have been obtained. Therefore we determined the urinary excretion of AAP, ALP, GGT, and NAG in renal-transplant recipients at different times after transplantation.1 We observed a dependence of urinary enzyme excretion on the interval after transplantation.

Materials, Methods, and Patients

**Apparatus:** Eppendorf photometer 1101 M and Eppendorf pipettes (Eppendorf Gerätebau, Netheler & Hinz, Hamburg, F.R.G.); pH-meter, Model MV 87 (VEB Präcticron, Dresden, G.D.R.); and chromatographic columns, 1 cm (i.d.) × 25 cm.

**Reagents:** 4-Nitrophenyl phosphate and Tris were obtained from Boehringer GmbH Mannheim, Mannheim, F.R.G.; alanine-4-nitroanilide, MgCl₂, NaCl, citric acid, diethanolamine (distilled before use) from E. Merck GmbH, Darmstadt, F.R.G.; Sephadex G50 (fine) from Pharmacia Fine Chemicals AB, Uppsala, Sweden; 4-nitrophenyl-N-acetyl-β-D-glucosaminide, and the test combination for GGT activity determination from Lachema, Brno, Czechoslovakia.

**Procedures:** AAP (5), ALP (6), and GGT (7) activities were measured at 405 nm and 37 °C by monitoring the increase in absorbance during 5 min. NAG activity was measured by a discontinuous procedure (8). Enzyme measurements were performed with urine samples that were prepared by gel filtration on Sephadex G50 (fine) (9). In the methods used, the final concentrations were: 2 mmol/L alanine-4-nitroanilide, 50 mmol/L Tri-HCl (pH 7.60) for AAP; 10 mmol/L 4-nitrophenyl phosphate, 0.5 mmol/L MgCl₂, 1 mol/L diethanolamine (pH 9.80) for ALP; 4 mmol/L γ-L-glutamyl-4-nitroanilide, 101 mmol/L NaCl, 101 mmol/L glycylglycine (pH 8.20) for GGT; and 2 mol/L 4-nitrophenyl-N-acetyl-β-D-glucosaminide, 100 mmol/L citrate buffer (pH 4.40), for NAG.

We adjusted the pH of solutions at 37 °C, using calibration buffers produced according to the U. S. National Bureau of Standards. The precision of enzyme activity determinations was controlled with Monitor II (Merz & Dade, Düdingen, Switzerland). Over an investigation period of 15 months, the following results (x ± 1 SD) were obtained: for AAP 14.9 ± 0.89 U/L, for ALP 46.2 ± 2.3 U/L, for GGT 57.1 ± 2.7 U/L, for NAG 19.3 ± 1.6 U/L.

**Patients and controls:** Urinary enzyme excretion was determined in two groups of recipients of cadaver renal allografts, who showed stable kidney function, had no urinary tract infections, and were not receiving nephrotoxic antibiotics. Aliquots of 24-h urine collections were stored at 4 °C until analyzed.

Group 1: 18 patients (seven women, 11 men; mean age 35.8, range ±13.3 years) in the early period after transplantation (mean, 1.80 months; range, 0.6 to 2.6 months) with a daily dose of prednisolone and azathioprine of 0.69 ± 0.20 mg/kg body weight and 1.44 ± 0.50 mg/kg body weight, respectively. The creatinine concentration in serum was 14.1 ± 5.8 mg/L.

Group 2: 18 patients (eight women, 10 men; mean age 37.4, range ±8.0 years) in the late period after transplantation (mean, 52 months; range, 14 to 140 months) with a daily dose of prednisolone and azathioprine of 0.27 ± 0.07 mg/kg body weight and 1.41 ± 0.66 mg/kg body weight, respectively, and a serum creatinine value of 11.7 ± 3.4 mg/L.

Control group: 15 healthy women and 13 healthy men (laboratory staff; age: 33.6 ± 5.2 years).

**Calculations:** Results were expressed in units (U) of enzyme activity excreted per gram of urinary creatinine. Statistical analyses were made with the nonparametric tests according to Mann–Whitney and according to Wilcoxon (10), because the values were not normally distributed. Correlation coefficients are given as rank correlation coefficients (r) according to Spearman (10).

**Results**

The two groups of renal-transplant recipients, comparable
zyme activity of merulonephritis, Discussion of generalization (18) of GGT, "p<0.01), excretion creatinine In reference over 2 months. Nevertheless, urinary excretion of AAP and NAG in group 2—i.e., later after renal transplantation—is lower than in group 1.

For more detailed information on the time-dependent behavior of urinary enzyme excretion in renal-transplant recipients, we determined urinary enzyme excretion in six patients during their attendance at our outpatient department over a period of 15 months after renal transplantation (Table 2). Thus we could use the individual values as the respective reference basis and eliminate the wide-inter-individual scatter in values. The statistical analysis with the matched-pairs signed-rank test according to Wilcoxon (10) showed there to be a significantly decreased excretion over this period, the temporal behavior of this decrease being different for each enzyme. Renal function, as reflected by the concentration of creatinine in serum, was unchanged during this period.

The investigations (n = 34) in these patients showed a weak but statistically significant correlation between the enzyme excretion and the time of determination after transplantation—for AAP (r = -0.444; p < 0.05), for GGT (r = -0.568; p < 0.01), and for NAG (r = 0.525; p < 0.01)—and also between enzyme excretion and the daily dose of prednisolone—for AAP (r = 0.762; p < 0.01), for GGT (r = 0.518; p < 0.01), and for NAG (r = 0.659; p < 0.01). The data show a general correlation between enzyme excretion and each of these two factors.

**Discussion**

If enzyme activity determinations in urine are to be validly used to detect rejection crises in the absence of other causes of renal damage (e.g., infection, medication, recurrent glomerulonephritis, renal artery stenosis) during outpatient control of renal-transplant recipients, two fundamental conditions have to be met: high analytical reliability in performing activity determinations over long periods of investigation, and long-term stability of the reference intervals of urinary enzyme excretion in renal-transplant recipients with well-functioning kidneys.

While the demand for high analytical reliability in determinations of urinary enzyme activities can be met today in the same way as in enzyme activity determinations in serum (11), there has been insufficient information so far on the long-term behavior of urinary enzyme excretion in renal-transplant recipients. However, detailed knowledge of the behavior of urinary enzyme excretion in renal-transplant recipients later than the first two months after renal transplantation is a definite prerequisite to judgments as to the long-term stability of individual reference values for urinary enzyme excretion.

Our study shows that the extent of urinary enzyme excretion in renal transplant recipients with no renal complications is a function of the interval after the renal transplant, and that values can be expected to be higher than in healthy persons.

The reasons for these findings may be manifold. During the early period after transplantation, repair processes in the transplanted kidney can induce a greater enzyme excretion. However, this would hardly explain the significant decrease of ALP and GGT excretion in the six patients continuously examined between the 9th and 15th month after transplantation, nor the higher excretion of AAP, ALP, and NAG in the urine of renal-transplant recipients in the late period as compared with the healthy control group. Apparently the prednisolone used for immunosuppression induces the synthesis of enzymes (12) in the kidney and thus an increased urinary enzyme excretion. As its dosage is gradually decreased, one might expect a decrease in enzyme urine excretion, a function of the interval after transplantation, the urinary enzyme excretion being, however, always higher than in healthy persons. The increased excretion due to inducing drugs would be based on enzyme induction in the kidney rather than on filtration of serum enzymes by the glomerulus (13).

Even though the reason for this interesting pathobiocochie phenomena is not exactly known, the practical conclusion is that for the individual renal-transplant recipient, urinary enzyme excretion will be related to the time elapsed after the renal transplant. Only when one allows for this fact, in the absence of other renal disorders, can one put sufficient

| Table 1. Urinary Enzyme Excretion by Renal-Transplant Recipients at Different Times after Transplantation, and in Healthy Persons * |
|-----------------|-----------------|-----------------|
| **Renal-transplant recipients** | **Control group** | **Group 1** | **Group 2** |
| AAP, U/g creatinine | 8.6 ± 2.4 | 29.5 ± 12.0 | 18.5 ± 7.8 |
| ALP, U/g creatinine | 5.1 ± 2.1 | 12.5 ± 6.1 | 13.5 ± 11.1 |
| GGT, U/g creatinine | 33.6 ± 7.0 | 41.8 ± 13.6 | 37.5 ± 10.8 |
| NAG, U/g creatinine | 3.6 ± 1.1 | 12.9 ± 6.2 | 10.6 ± 4.7 |
| * Group 1: 18 renal transplant recipients in the early period after transplantation (1.6 months after transplantation). Group 2: 18 renal transplant recipients in the late period after transplantation (52 months after transplantation). Control group: 26 healthy persons. Data are mean values ± 1SD. Differences were calculated by nonparametric test according to Mann–Whitney. n.s., not significant. |

| Table 2. Time-Dependent Behavior of Urinary Enzyme Excretion in Six Renal-Transplant Recipients after Transplantation * |
|-----------------|-----------------|-----------------|
| **Months after transplantation** | **2** | **9** | **15** |
| AAP, U/g creatinine | 47.4 ± 7.4 | 20.0 ± 7.8 | 16.4 ± 8.2 |
| ALP, U/g creatinine | 23.0 ± 14.5 | 14.8 ± 6.2 | 8.7 ± 3.3 |
| GGT, U/g creatinine | 57.3 ± 17.8 | 59.7 ± 30.9 | 33.0 ± 20.1 |
| NAG, U/g creatinine | 22.2 ± 15.7 | 11.4 ± 4.8 | 5.1 ± 10.9 |
| Creatinine in serum, mg/L | 16.6 ± 5.3 | 15.5 ± 4.5 | 16.3 ± 5.4 |
| * Data are given as mean values ± 1 SD. Significance of differences was calculated by nonparametric test according to Wilcoxon. n.s., not significant. |
diagnostic reliability in urinary enzyme excretion as an early predictor of rejection in the late period after renal transplantation.

References