# Calcineurin phosphatase activity measurements in renal transplant patients treated with cyclosporine and tacrolimus

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ABSTRACT

The calcineurin inhibitors cyclosporine (CsA) and tacrolimus (FK) are the most commonly used immunosuppressive drugs in transplant medicines today. The patients are monitored with classical pharmacokinetic methods, such as trough-level measurements and $C_2$ measurements (drug concentration two hours post dose), but despite concentrations within the therapeutic window, patients still exhibit both rejection and toxicity.

This PhD dissertation describes the development and usage of a pharmacodynamic assay that determines calcineurin phosphatase (CaN) activity in renal transplant patients treated with cyclosporine or tacrolimus.

Basal enzyme kinetic experiments confirmed that it was possible to measure CaN activity with this assay. Furthermore it was demonstrated that the enzyme did not exhibit circadian variation.

CaN activity in 20 renal transplant patients treated with FK on day three and 14 after transplantation was investigated. The patients had blood samples drawn before and one, two, three, four, and six hours after oral intake of FK. We demonstrated an inverse relation between FK concentrations and CaN activity. Furthermore, we found the enzyme activity to be significantly inhibited at one, two, and three hours compared with pre-dose level.

In study IV, we investigated the CaN activity in 40 renal transplant patients with stable allograft function three times in a 6-month-period. The most important finding was the fact that CsA and FK display different CaN inhibitory profiles. Surprisingly, the FK-treated patients were found to have only just-significant inhibition of CaN activity compared with the CsA-treated patients that displayed a significant inhibition profile as that seen in recently transplanted patients. By comparing the results from study IV and study II, we found that the renal transplant patients with stable allograft function have the same level of CaN activity, as did the healthy non-medicated subjects. These results raise the question whether CaN is up-regulated in renal transplant patients treated with CaN inhibitors and also whether CaN inhibition is crucial for maintaining graft function in patients with stable allograft function. The CaN inhibition in these patients could reflect only the side effects of the drugs.

In conclusion, the relatively large coefficients of variation of the method indicate that this assay will be inadequate for use as a sole monitoring tool for an individual patient. This assay must be improved through further investigations before this could be the case. Nonetheless, the CaN assay can be a useful scientific tool toward a deeper understanding of the CaN inhibition in various groups of renal transplant patients. It is our hope that the method can contribute to the already growing knowledge about the immunosuppressive actions of the CaN inhibitors.