In conclusion real-time PCR followed by melting curve analysis is a rapid, simple, accurate method for genotyping the CYP2D6*6 allele.

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Monitoring of calcineurin activity under controlled systemic cyclosporine exposure after renal transplantation

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The calcineurin inhibitors (CNI) cyclosporine (CsA) and tacrolimus (TRL) are potent immunosuppressive drugs that are extensively used in organ transplantation. Unfortunately, severe adverse drug effects such as nephrotoxicity, diabetes mellitus, malignancies and cardiovascular toxicity are observed in CNI treated patients. Since large inter-individual variation in CNI pharmacokinetics is observed, therapeutic drug monitoring is required to control the therapeutic index. In practice, blood concentration of TRL and CsA is monitored, but since pharmacokinetic monitoring is a surrogate of effect, measurement of effect (pharmacodynamics) provides, at least in theory, a more accurate marker (1). We have therefore developed a calcineurin assay (2) and in this study we monitored calcineurin activity in renal transplant patients that were treated with CsA.

Methods

Forty-seven renal transplantation patients were monitored for leukocyte CN activity and CsA blood concentration. All patients received quadruple immune suppression including CsA, prednisolone, mycophenolate sodium and basiliximab prophylaxis. Samples were taken before transplantation and 2 weeks, 6 weeks, 6 months after graft implantation. After transplantation samples were taken before drug intake (12 hours after previous dose) and one patient was monitored for several hours after drug intake. CN leukocyte activity was measured as previously described (2) and CsA blood concentrations were measured using a fluorescence polarization immunoassay on an Abbott AxSYM system. CsA exposure was $AUC_{0\text{-}12}$ controlled and aimed at 5400 h*µg/L the first 6 weeks after transplantation and 3250 h*µg/L thereafter (3). Sudent's t-tests were performed to test significance and statistical significance was defined as $p < 0.05$.

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Results

An overview of the measured CN activities and CsA blood concentrations is found in table 1. When pre-transplantation (without CsA) leukocyte CN activities were compared to T0 CN activities, lower CN activities were found on week 2 (p=0.0003) and month 6 (p=0.02), but not on week 6 (p=0.2). A large spread in CN activities was observed between patients: coefficients of variations were 38%, 32%, 56% and 31% for Pre-Tx, week 2 (T0), week 6 (T0) and month 6 (T0) respectively. When a single patient is monitored in time for CN activity an inverse relation between CsA concentration and CN activity is found for the renal

Table 1. CN activity and CsA concentration in renal transplantation patients. Mean ± SD values of CN activities and CsA concentrations observed in renal transplantation patients just before drug intake (12 hours after previous dose). * CN activity is expressed as pmol · min^{-1} per 1 million leukocytes.

	CN activity*	[CsA] in µg/L
Pre-Tx	220 ± 84	
Week 2	153 ± 49	292 ± 108
Week 6	188 ± 106	228 ± 67
Month 6	149 ± 47	120 ± 38

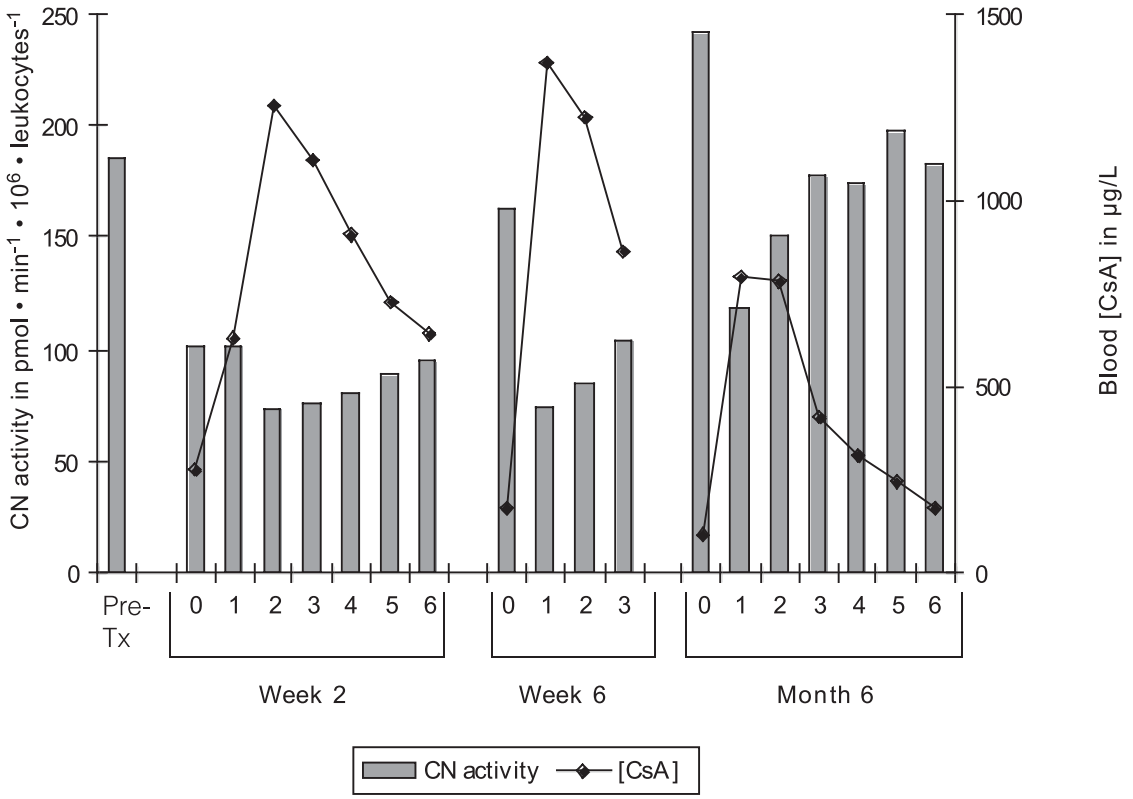

Figure 1. Pharmacodynamic monitoring of a renal transplantation patient. Leukocyte CN activity and blood CsA concentration in time just before and after drug intake. Numbers represent hours after drug intake (0 is just before drug intake).

transplantation patient. After drug intake a partial and temporal CN inhibition is observed and a clear relation between blood CsA concentration and CN activity is visible, see figure 1 and 2.

Discussion

Pharmacodynamic monitoring of CNI is an interesting new tool to improve CNI therapy; reducing side-effect occurrence, while maintaining successful immune suppression, since it theoretically provides more insight in immune suppressive status. Here we show that monitoring leukocyte CN activities is feasible in clinical practice. CN inhibition by CsA in CsA treated patients is reflected in leukocyte CN activities. CN inhibition in renal transplant patients treated with CsA is only partial and has a temporal profile, which seems to be sufficient for successful immune-suppression. Within our patient group a large variation in CN activity is observed, though systemic CsA availability was AUC controlled. This variation could provide therapeutic information, however for further understanding of CN activities in renal transplantation patients a comprehensive clinical evaluation is required.

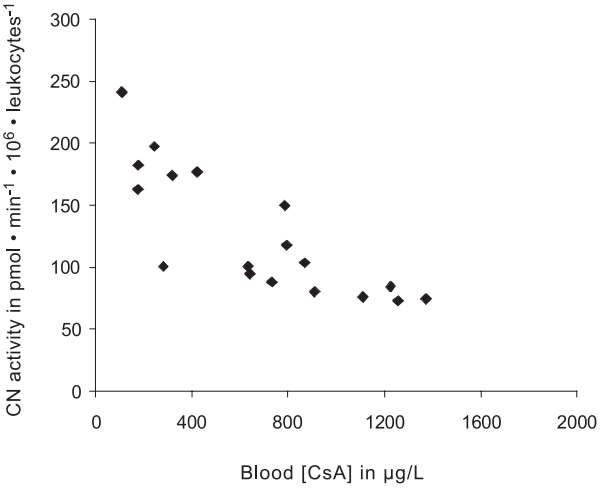

Figure 2. Pharmacokinetic / pharmacodynamic relation of CsA concentration and CN activity for renal transplantation patient.

Conclusion

The investigation of CN activity is feasible in clinical practice. Despite controlled systemic drug exposure, a wide variation in CN activity was observed. Further analysis of the determinants of variation in calcineurin activity could provide a more precise tool to define and maintain the therapeutic index of CNI therapy after organ transplantation.

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