Is Calcineurin Activity Useful as a Biomarker to Optimize Cyclosporine A Therapy in Renal Transplant Recipients?

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Introduction

- Acute rejection episodes and clinical nephrotoxicity relate to cyclosporine A (CsA) exposure in renal transplant recipients [1].
- Inter-individual variability in CsA exposure is high.
- Therapeutic drug monitoring (TDM), using a concentration biomarker, is essential.
- Despite routine TDM, still patients suffer from acute rejection episodes and acute and chronic nephrotoxicity.
- Insight into the individual susceptibility for CsA therapy is warranted.
- A response biomarker could be used to explain differences in clinical response between patients [2].
- Measurement of the phosphatase-activity of calcineurin, the target enzyme of CsA, could be used as a biomarker.
- The pharmacokinetic-pharmacodynamic (PK-PD) relationship between CsA exposure and the activity of the calcineurin enzyme was evaluated.

Aim

The aim was to determine whether this biomarker reflects between patient variability in treatment effect of CsA.

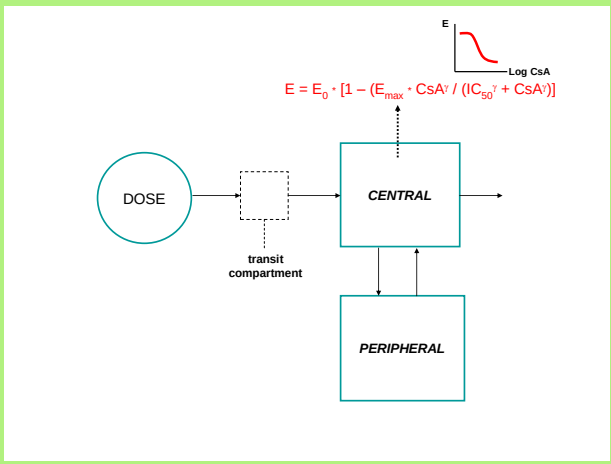


Figure 1. PK-PD model

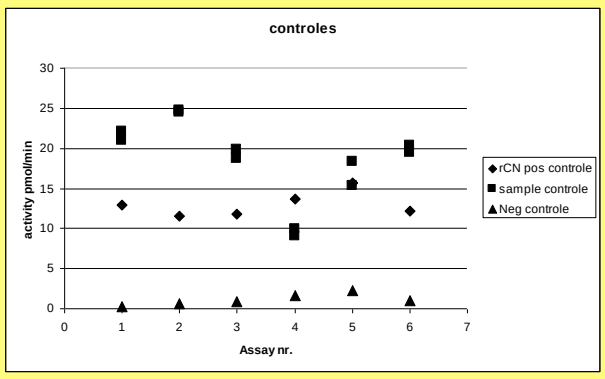


Figure 5. Plot of the samples used as controls for calcineurin activity measurements in this study. The negative control ('Neg.') and the recombinant calcineurin enzyme control sample ('rCN') are used routinely. In addition, we added a patient control sample ('sample control') to exclude assay variability issues. Despite low variability in the recombinant control, we did observe large variability with an aliquotted patient control sample. This indicates large variability in the assay (reproducibility problem) and serves as a potential explanation for the observed within-subject variability in baseline activity.

Methods

Renal transplant recipients (n=95) were followed for 6 months after transplantation and received basiliximab, mycophenolate-sodium, prednisolone and CsA. The initial CsA dose of 4 mg/kg b.i.d was adjusted to a preset target AUC. CsA concentrations were measured together with calcineurin activity in the mornings of weeks 1, 4, 8, 16 and 26, of which weeks 1 and 26 were densely sampled. On the other occasions a limited sampling strategy was applied, with measurements on t = 0, 2 and 3 h to estimate the AUC of CsA as described by Cremers *et al*.[3]

Calcineurin phosphatase activity was determined in the white blood cell fraction with a spectrophotometric-assay based on phosphate quantification as described by Sellar *et al*.[4]. CsA concentrations were determined in whole blood with an immunoassay (FPIA-Axsym-Abbott). Non-linear-mixed-effects-modelling was used for data analysis (NONMEM, version VI, Icon Development solutions, Ellicott City, Maryland, USA) and a sequential PK-PD analysis was performed. Moreover, a series of covariates were collected to evaluate their effect on the PK and PD. These covariates include demographics (*i.e.* bodyweight), prednisolone dose, white blood cell differentiation (mono-, lympho-, granulocytes (baso-, neutro-, eosinophil)), amount of intracellular protein, and creatinin values.

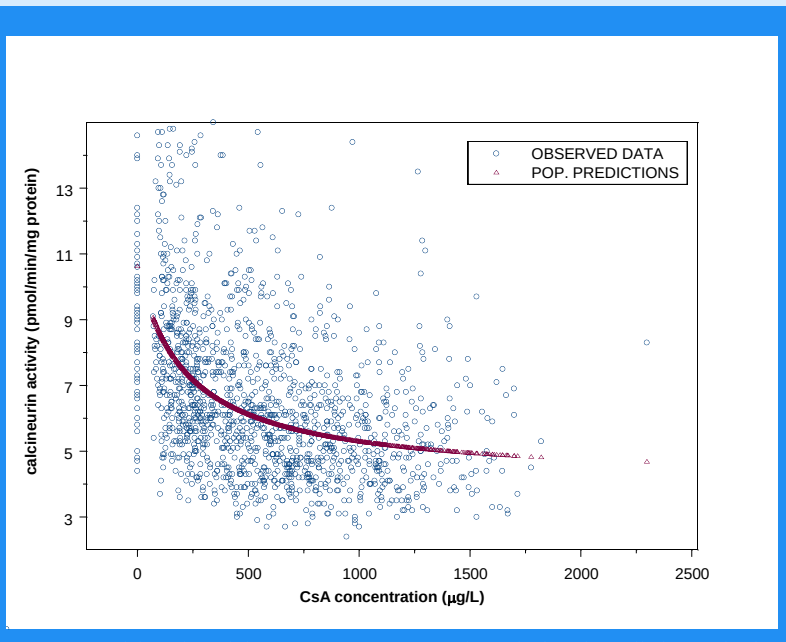


Figure 2. Calcineurin activity *versus* CsA concentration. Displayed are all observed values and the population predicted values for the calcineurin activity.

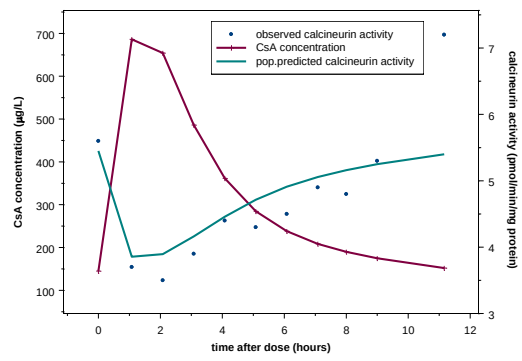
Figure 3. CsA concentration and calcineurin activity *versus* time after dose (top plot on the right) and concentration *versus* effect curve (middle plot on the right) obtained from 1 individual at 6 months after transplantation.

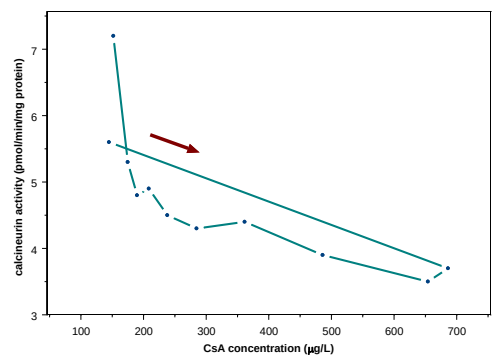
Results & Discussion

- The relationship between CsA concentration and calcineurin activity could be described by a two compartment model with delayed absorption and a direct effect as depicted in Fig. 1-3.
- Prednisolone and body weight were included in the model as covariates for CsA clearance.
- Baseline calcineurin activity (E_0) was 14 pmol/min/mg protein (median), but showed considerable within subject variability of 28% (Fig. 4).
- Maximum inhibition (E_{max}) was 48% of the baseline activity, IC_{50} was 223 µg/L and the Hill factor was 1.7.
- Within subject variability (inter-occasion variability) in the baseline hampered the identification of between subject variability in E_{max} and IC_{50}.
- Inter-occasion variability could result from the biological system itself, from the calcineurin assay or it could be related to variability in the composition of the white blood cell sample.
- White blood cell subsets (*i.e.* monocytes *versus* lymphocytes) have a different activity of calcineurin [5]. This is reflected by different protein concentrations in the cell. The covariate intracellular protein was linearly related to baseline activity (E_0) with a decrease of 1.6% upon a 10 mg increase in amount of protein.
- A recombinant control sample and a validated assay were used, while variability in the obtained assay results was observed (Fig. 5).
- Assay should be optimized by improving the sample preparation procedure or by measuring calcineurin activity at the target site, *i.e.* calcineurin activity in T-cells.

Conclusions

A clear relationship between CsA concentration and calcineurin activity was observed. However, the variability in the response biomarker between occasions was too large to identify between-patient variability in efficacy and potency of CsA to inhibit calcineurin. Therefore, between patient variability in treatment effect of CsA could not be related to this biomarker.





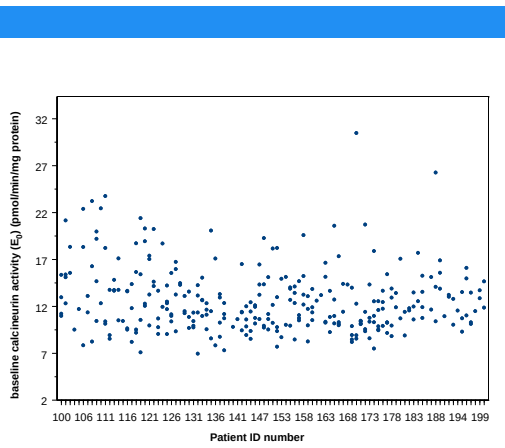


Figure 4. Within-subject variability in baseline activity (E_0).

References

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