Pharmacodynamic strategy for monitoring the extent of immunosuppression: Requirement of a kinase inhibitor for measuring calcineurin phosphatase activity

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The optimal balance between a too strong or a too weak immunosuppression is difficult to achieve following transplantation. An inadequate immunosuppression with insufficient therapy may lead to rejection whereas excessive immunosuppression would facilitate the development of cancer and infections. The current monitoring of immunosuppression, which is performed through immunosuppressant blood levels, is not satisfactory. A pharmacodynamic approach, based on the measurement of the effect of the immunosuppressants on their cellular targets, such as calcineurin activity for the inhibitors of calcineurin, has been developed with the aim to reduce the incidence and severity of post-transplant complications. The determination of calcineurin activity consists in the direct measurement of the dephosphorylation of a phosphorylated substrate of calcineurin, the RII peptide.

The present study investigates the possibility of rephosphorylation of the calcineurin substrate and its influence on a pharmacodynamic strategy for monitoring the extent of immunosuppression following transplantation. To do so, kinetics experiments were realized in the presence of various kinase inhibitors and a significant endogenous kinase activity, taking place during the enzymatic assay, was found. Moreover, a high variation in kinase activity was observed among patients and over time, with subsequent alterations in the measurement of calcineurin activity. Therefore, we developed and validated a LC-MS/MS assay for measuring calcineurin activity in biological samples wherein a kinase inhibitor is present in the assay reaction mix. This assay uses a simple sample preparation and a stable isotope of the susbstrate as internal standard. The linear range for the dephosphorylated substrate was 0.03-3.0 µM, and the LOQ was of 0.02 µM. All relative standard deviation and relative error values obtained in intra- and inter-day analyses were below 10%. Furthermore, the study of the stability of calcineurin activity showed that blood samples could be stored up to 4 days at 4°C before being processed.

In conclusion, we developed and validated a robust, sensitive and specific LC-MS/MS method for measuring calcineurin activity. This method could be used in clinical studies to

routinely monitor calcineurin activity as a potential biomarker of post-transplant complications.