Poster

[P25-6] P25-6: Immunosuppressive drugs (1): LC-MS/MS assay

Chair: Tsutomu Nakamura, Japan

Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall (1F)

(Mon. Sep 25, 2017 12:30 PM - 1:30 PM Annex Hall)

[P25-6-2] A validated UPLC-MS/MS method for intracellular tacrolimus concentrations

Brenda C. M. de Winter[1], Soma Bahmany[2], Elly L.E.A. De Wit[3], Dennis A. Hesselink[4], Teun van Gelder[5], Nauras M. Shuker[6], Bart C.H. Van Der Nagel[7], Birgit C.P. Koch[8] (1.Erasmus MC, 2.Erasmus MC, 3.Erasmus MC, 4.Erasmus MC, 5.Erasmus MC, 6.Erasmus MC, 7.Erasmus MC, 8.Erasmus MC)

Keywords: Tacrolimus, intracellular, peripheral blood mononuclear cells

Background

After solid organ transplantation, tacrolimus in combination with mycophenolic acid is given to prevent rejection. Therapeutic drug monitoring is used to reach target trough concentrations of tacrolimus in whole blood. Underexposure is associated with acute rejection, whereas overexposure is associated with an increased risk of toxicity. Because the site of action of tacrolimus is the lymphocyte, and the fact that tacrolimus binds for approximately 80% to the erythrocyte, the intracellular tacrolimus concentration in lymphocytes is possibly more relevant than whole blood concentrations in predicting treatment efficacy. For this purpose we aimed to develop and validate an UPLC-MS/MS method to measure tacrolimus concentrations in isolated peripheral blood mononuclear cells (PBMCs).

Methods

PBMCs were isolated from whole blood using a Ficoll separation technique. A cell suspension of 50 L containing 1 million PBMCs was used in combination with MagSiMUS-TDM^{PREP} (MagnaMedics) to determine the tacrolimus concentration. To each sample we added: 30 L lysis buffer, 20 L reconstitution buffer containing 13C-H4-tacrolimus as internal standard, 40 L MagSiMUS-TDM^{PREP} Type I Particle Mix and 175 L Organic Precipitation Reagent VI for methanol-based protein precipitation. After centrifugation 10 L of the supernatant was injected into the UPLC-MS/MS using a Waters Acquity BEH C18 1.7 μm (2.1 mm ×50 mm) column, with a gradient of water and methanol (both with 2mM ammonium acetate and formic acid).

Results

The method was validated resulting in high sensitivity and specificity (LLOQ is 0.5 pg/1 million PBMCs). The method was linear (r^2=0.997) over the range of 0.5-125 pg/million PBMCs. The inaccuracy was <5% and the imprecision was <15%. The washing steps following Ficoll isolation could be performed at room temperature or on ice, no effect on the results was seen. The ratio between intracellular and whole blood tacrolimus levels differed between patients, but was stable within one patient in 4 samples over a range between 0-8h after dose administration.

Conclusions

A method for the analysis of tacrolimus concentrations in PBMCs was developed and validated for use in clinical care. Further research will be performed to investigate the correlation between concentrations in PBMCs and clinical outcome.