Poster

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

Chair: Tsutomu Nakamura, Japan

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[P25-6-4] UHPLC-MS/MS based determination of cyclosporine A, tacrolimus and everolimus in autologous serum eye drops for the treatment of ocular manifestation of GvHD in patients following allogeneic hematopoietic stem cell transplantation

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Keywords: autologous serum eye drops, ocular GvHD, calcineurin inhibitors, everolimus, LC-MS/MS

Background

Graft-versus host disease (GvHD) is a leading cause of morbidity and mortality in patients who underwent allogeneic hematopoietic stem cell transplantation (aHSCT). Treatment regimens with immunosuppressive agents such as mycophenolic acid (MPA), cyclosporine A (CsA), tacrolimus (Tac), and everolimus (Eve) form the basis of GvHD management in addition to corticosteroids. Ocular GvHD manifestations commonly lead to pathological changes in the anterior segment of the eye, manifesting as dry eye disease and severe wound healing deficiencies of the cornea. Allogeneic and autologous serum eye drops (ASED) have been reported as effective treatment options for ocular GvHD. At present, it remains unclear if immunosuppressants can be found as constituents in ASED manufactured for patients with ocular GvHD and concomitant immunosuppressive therapy.

Methods

ASED were manufactured for patients with ocular GvHD according to a previously published protocol at the Institute of Transfusion Medicine, University Hospital of Cologne using a closed system. Residual serum samples within the production system were labeled and stored at -80˚C. A UHPLC-MS/MS method was developed and validated enabling simultaneous determination of CsA, Tac, and Eve in serum matrix. Extraction of serum samples was based on protein precipitation with acetonitrile. Stable isotope labeled analogues ($^{2}H_{12}$-cyclosporine A, $^{13}C,^{2}H_{2}$-tacrolimus, $^{13}C_{2},^{2}H_{4}$-everolimus) were employed as internal standards for quantitative analysis.

Results

Linearity was demonstrated from 10-600g/L for CsA and from 1-50g/L for Tac and Eve, respectively (R^2 >0.99). Chromatographic separation was achieved using a C18 column (50x2.1mm, 1.9m particle size) and a gradient elution. Retention time ranged from 3.39-3.77min, and total run time was 5.0min. For all analytes, inter- and intra-day precisions (CV, %) varied between 2.0 and 11.3 and inter- and intra-day trueness were within 10.7% of true values. The lower limits of detection and quantification were 0.5, 0.2, and 0.2 and 1.6, 0.5, and 0.6 g/L for CsA, Tac, and Eve, respectively.

Conclusions

The established UHPLC-MS/MS method can be applied to resolve the question if immunosuppressive drugs are present in ASED manufactured from serum of patients with ocular GvHD and concomitant immunosuppressive therapy. Future studies are currently designed to investigate positive or negative

influence of ASED-derived immunosuppressive drugs on ocular surface integrity.