Poster

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

Chair: Tsutomu Nakamura, Japan

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[P25-6-9] Investigation of tacrolimus transport mechanisms into the white blood cells: Does only P-glycoprotein matter?

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Background

Measuring tacrolimus (TAC) concentration in peripheral blood mononuclear cells (PBMC), closer to the target cells, have been suggested to refine TAC therapeutic drug monitoring since it could better reflect the drug effect than whole blood concentrations. Polymorphisms of the efflux protein P-glycoprotein (Pgp) have been reported to influence TAC concentrations in PBMC. Nevertheless, mechanisms influencing TAC diffusion into PBMC are not well characterized and data regarding other membrane transporters involved in TAC cellular distribution are sparse.

This work aimed at describing, *in vitro,* the kinetics of diffusion of TAC into PBMC and investigating the contribution of Pgp to regulate TAC intracellular concentration.

Methods

PBMC were isolated from healthy donors whole blood using density gradient device (Stemcell). Cells were incubated in RPMI medium under agitation with various concentrations of TAC (250,500 and 1000 pg/millions of cells respectively) for 5 min to 4h and under three experimental conditions: 37˚C (physiological conditions) 4˚C (inhibition of influx and efflux active transport), 37˚C + 40 M verapamil (inhibition of Pgp). After incubation, supernatants of cells suspensions were discarded, and intra-PBMC TAC concentration was determined using liquid chromatography mass spectrometry. The experiments were performed in hexaplicate.

Results

TAC intra-PBMC concentration was maximal after 1h of incubation at 37˚C and after 15 min for samples at 4˚C Then, concentrations decreased of 25% after 4h of incubation. No statistical difference was found between TAC intracellular concentration incubated with or without verapamil. Mean TAC PMBC concentrations were 60% lower in samples incubated at 4˚C compared to the 37˚C groups.

Conclusions

These results provide interesting mechanistic data and highlight that active influx transporters might be involved in TAC uptake by the PBMC. Moreover, because verapamil (Pgp inhibitor) failed to increase TAC concentration in cells, it is likely that other drug efflux transporters than Pgp regulate TAC concentrations in PBMC. Further experiments are ongoing to identify these transporters and confirm these hypotheses to improve our understanding of TAC intracellular pharmacokinetics.