Characterisation of tacrolimus demethylated metabolites using high resolution mass spectrometry: an open ring structure?

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Background:

The pharmacodynamics and toxicity of tacrolimus (TAC), a widely used immunosuppressant, are well documented. However, the part played by its metabolites is poorly understood. As a first step, this study aimed to revisit TAC metabolites (TM) using liquid chromatography (LC) coupled with high resolution mass spectrometry (MS).

Method:

The kinetics of TAC metabolization was investigated *in vitro* using human liver microsomes and clinically in kidney transplant patients. TM were extracted from the incubation medium or patient blood using Quechers salts. The analysis was carried out on an LC/Q-TOF MS (TripleTOF® 5600+) using information dependent acquisition (IDA) and multiple reaction monitoring at high resolution (MRM^{HR}) methods.

Findings:

During metabolization of TAC, we observed a new compound (NTAC) eluting earlier than TAC and with the same m/z. When compared with TAC fragmentation pattern, we found seven supplemental fragments in the NTAC EPI spectrum suggesting a conformation change. We also found four demethylated TAC (DMT), and four di-demethylated TAC (DDMT), already reported by Iwasaki respectively as M-I/M-III and M-V/M-VI/M-VII (1). All these metabolites presented an EPI spectrum similar to that of NTAC, except for a chemical shift corresponding to the loss of one or two methyl groups. In addition, a fifth DMT, corresponding to M-II, presented an EPI spectrum similar to that of TAC with a loss of methyl group was observed. Based on the different structures of TAC found in the literature and the fragmentation of NTAC found here, the best matching conformation for us is an open ring structure (ORS) at the C14 position with 2 OH groups at the C10 position. This would mean that M-I, M-III, M-V, M-VI and M-VII have an ORS, while M-II conserves the original conformation. Moreover, two DMT were found in patient blood with an EPI spectrum suggesting an ORS.

Conclusion:

Except for M-II, all DMT may have an ORS. This could explain their lack of immunosuppressive activity, whereas M-II conserves it. Molecular dynamics simulations are still in progress in order to confirm this hypothesis.

Key words:

Tacrolimus, metabolites, Q-TOF, open ring structure

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