Introduction

- Maribobufagenin (MBG), a cardiotonic bufadienolide, is a selective inhibitor of the α1 subunit of Na+,K+-ATPase. MBG is mainly known due to its role as the major cardiotonic steroid in the Bufo Marinus venom located in the parotoid gland secretions.
- Due to its vasocostrictive, cardiotonic and natriuretic activities, endogenous MBG is implicated in volume expansion-mediated hypertensive states such as preeclampsia. Increased plasma MBG has been observed in preeclamptic women and a rat model for preeclampsia (PE) [1-3]. The increased MBG production seems to appear prior to the development of the symptoms, leading us to propose MBG as a potential biomarker for PE.
- This hypothesis demonstrates the need for an accurate and sensitive analytical method for MBG plasma levels quantification in human. A LC-MS/MS based assay designed to determine MBG in human plasma is being optimized and focuses on our main target: to reach the lowest limit of quantification.
- Currently, only maribobufagenin-like material using poor-specific immunoassays has been found in humans [4,5]. Here we report the identification of MBG in non-pregnant human plasma as well as in a plasma sample obtained from a 15 weeks pregnant woman using a LC-MS/MS assay, opening the perspective of investigating the potential of MBG in preeclampsia risk assessment.

Results

1) Extraction of MBG from Bufo marinus venom

- Given that no MBG standard is commercially available, we needed to develop an effective extraction and purification method to acquire the reference compound.
- Crystallized Bufo marinus venom was analyzed in order to confirm the presence of MBG in the toad venom using TLC-MS and LC-MS. After optimization of an extraction method, the identity of purified MBG has been confirmed by TLC-MS and mass spectrometry.

2) Plasma Extraction process

- SLE process on 96-well plates has been developed and provides selective MBG and IS extraction from plasma with a 50% recovery for MBG.

3) MS/MS characterization in human plasma: 1st elaboration

At present, only MBG-like material has been determined in human samples using poor-specific immunoassays. Using the purified MBG, a sensitive MRM based LC-MS/MS assay was developed for MBG. Preliminary tests showed that MBG could be easily detected at 250 ng/mL using 4 mass transitions. Using 2 specific MRM transitions (a quantifier one and a qualifier one), the LC-MS/MS assay allowed us to detect endogenous MBG in both plasma obtained from healthy non pregnant volunteers and from 3 different 15 weeks pregnant women (n=3).

4) LC-MS/MS method development: 2nd elaboration

The optimization of the assay allowed us to quantify MBG at 50 pg/mL with S/N > 10 and to identify endogenous MBG in a 21 weeks pregnant woman plasma sample after extraction.

Conclusion

- We obtained pure MBG as a standard for analytical method development following extraction of MBG from Bufo marinosus crystallized venom and subsequent purification.
- A preliminary SPE clean-up step for MBG and the Internal Standard, 5α-dihydrotestosterone-d3, from human plasma has been set up with an extraction yield of 51% for MBG.
- A first sensitive LC-MS/MS assay developed at Metabolomic Diagnostics allowed us to authenticate MBG in human plasma. MBG could be identified in non-pregnant healthy patients as well as in early pregnant (15 weeks) volunteers.
- The LOQ obtained by the 2nd elaborated method (50 pg/mL) fully satisfies the need for quantification of MBG plasma levels in pregnancy (+/- 100 pg/mL range). The quantification method based on the response factor approach will be validated thanks to the accuracy profiles strategy. A primary observational clinical study in pregnant women with non-pregnant controls is now under design and will allow us to confirm previous results observed in case of PE.