Identification of marinobufagenin in plasma as a promising LC-MS assay for preeclampsia risk assessment

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Introduction

Marinobufagenin (MBG), a cardiotonic bufadienolide, is a selective inhibitor of the α1 subunit of Na+-K+-ATPase. Bufadienolides are mainly located in the parotid gland secretions of some toad species but can also be found in mammals.

- 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 appears prior to the development of the symptoms, leading us to consider MBG as a biomarker for PE.
- This hypothesis involves an accurate and sensitive analytical method for MBG plasma levels quantification in order to further investigate the implications of MBG in PE. Currently, only marinobufagenin-like material has been found in humans. 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 utilising a LC-MS assay, opening the perspective of investigating the potential of MBG in preeclampsia risk assessment.

Materials and Methods

**1) Venom Collection**

- Venoms were obtained from Bufo Marinus, a toad commonly found in Belgium. Freshly collected venom was kept at -20°C until use.
- After opening the glands, the secretion was collected by squeezing them with a force of 40 Newtons.
- The crude venom was then filtered through a 0.22 μm filter and stored at -20°C until use.

**2) Extraction and identification of MBG in Bufo Marinus venom**

- Extraction was performed using standard SPE cartridges (C. Lenaerts et al., 2014).
- MBG stock solution was prepared in 100% MeOH.
- HPLC-UV and MS conditions were used for the analysis.
- Identification was confirmed by LC-MS/MS conditions.

**MBG characterization in human plasma**

**1) Solid Phase Extraction (SPE) process**

The setup of a sensitive quantitative method for MBG plasma levels starts with an extraction from plasma samples by SPE. This preliminary step is essential for sample clean up and concentration. Several SPE sorbent phases were tested: clean up with Waters® HLB (hydrophilic lipophilic balanced) cartridge gave the best extraction yield (92%) for a relatively clean sample.

**2) MS/MS characterization in human plasma: developed in Metabolomic Diagnostics**

Currently, only MBG-like material has been determined in human samples using two different immunoassays, but for both the results were distorted due to cross-reactivity [4,5]. 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 0.25 ng/mL. The LC-MS/MS assay allowed us to detect endogenous MBG in both plasma obtained from healthy non pregnant volunteers and from a 15 weeks pregnant woman.

**Conclusion**

- We obtained pure MBG as a standard for analytical method development following extraction of MBG from Bufo Marinus crystalized venom and subsequent purification.
- A SPE sample clean-up step for MBG from human plasma has been developed with an extraction yield of 92%.
- A sensitive LC-MS/MS assay was developed which 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.
- With MBG plasma levels being reported as increased when preeclampsia manifests, this dosage method once fully developed will help to quantify MBG plasma levels in early pregnancy, giving the clinicians a promising opportunity to assess the potential of MBG for early preeclampsia risk assessment. In addition the availability of an LC-MS/MS method will help the elucidation of some research questions such as: the biosynthetic origin of MBG and/or what role MBG exactly plays in the PE syndrome.

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