

Marinobufagenin as a promising preeclampsia risk assessment marker: purification from toad venom and LC-MS identification in human plasma

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Introduction

Marinobufagenin (MBG) is an endogenous bufadienolide cardiac inotrope which is demonstrating growing interest in the early diagnosis of volume expansion-mediated hypertensive states such as preeclampsia (PE) and end-stage renal disease hypertension.

Mammalian MBG is an inhibitor of the $\alpha 1$ isoform of Na^+, K^+ -ATPase with vasoconstrictive and cardiotoxic properties, resulting in hypertension and natriuresis. Elevated endogenous MBG levels have been described in pregnant mammals and especially in preeclamptic patients [1-3]. The rise of endogenous MBG appears prior the development of the main symptoms of PE, leading us to consider MBG as one of the potential target in the biomarker panel for PE.

A sensitive and accurate analytical method is needed to assess MBG in as lower level as possible in plasma. Currently, only marinobufagenin-like material has been found in humans using two published quantification methods based each on immunoassays [4,5]. These techniques suffer from a lack of specificity due to cross-reactivity and tend to exhibit high variability at low concentrations [6].

Objective

Our aim is to develop a MBG assay using a more specific and easy to access technique, such as LC-MS/MS. An algorithm dealing with the MBG plasma levels might be established by clinicians in the future, in order to predict, in combination with other clinical and biological markers, the risk for preeclampsia in pregnant women.

Methods

As the major source for MBG is located in the parotid glands of the *Bufo Marinus* toad, we developed a purification method from toad venom in order to get pure MBG standard.

A pre-extraction procedure was elaborated to concentrate and clean the plasma sample prior to its analysis.

A LC-MS based assay designed to determine MBG in human plasma is being optimized, giving us the opportunity to investigate MBG in non-pregnant healthy volunteers plasma as well as in early pregnant women plasma.

Results and discussion

Pure MBG has been successfully extracted from the *Bufo Marinus* toad venom and the identity of the compound has been confirmed.

An extraction procedure for MBG from plasma has been set up by use of solid phase extraction cartridges.

Preliminary results allowed us to authenticate the presence of MBG by LC-MS/MS in non-pregnant women as well as in early pregnancy.

Further optimization and validation of the LC-MS assay are needed to quantify MBG plasma levels. However, these pioneering observations, are giving the clinicians a promising perspective for early preeclampsia risk assessment in pregnant women.

References

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