Elaboration of a quantification assay of marinobufagenin in human plasma: a novel approach based on response factor quantification by LC-MS/MS

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Marinobufagenin (MBG) is a bufadienolide cardiac inotrope implicated in the early diagnosis of volume expansion-mediated hypertensive states such as preeclampsia (PE).

Endogenous MBG is an inhibitor of the α1 isoform of Na^+,K^+-ATPase with vasoconstrictive and cardiotonic 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 seems to appear prior the development of the main symptoms of PE, leading us to consider MBG as one of the potential biomarkers for PE.

This demonstrates the need for a sensitive analytical method to detect MBG in plasma at low levels. Currently, the enhanced production of MBG in preeclamptic patients has been described using poor-specific immunoassays based on the detection of marinobufagenin-like material [4,5]. These techniques suffer from a lack of specificity due to cross-reactivity and tend to exhibit high variability at low concentrations [6]. Moreover, the two studies that recorded MBG plasma levels in preeclampsia compared to normal pregnancy are in marked discrepancy concerning the values of MBG plasma concentration.

Our aim is to develop a specific and sensitive analytical MBG assay by LC-MS/MS with special attention to the limit of quantification (LOQ). An algorithm dealing with the MBG plasma levels might be established in the future, in order to help for prediction of the risk for preeclampsia in pregnant women.

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. The identity of the compound was confirmed using TLC-MS and HPLC-MS/MS.

A very sensitive and specific LC-MS/MS based assay is now being optimized in order to determine MBG in human plasma. The assay is preceded by an extraction step of the plasma on SLE cartridges. Using 5α-dihydrotestosterone-d_3 as internal standard for response factor calculation, the obtained LOQ fully satisfies the need for quantification of MBG plasma levels in pregnancy (nmol/L range). This assay allowed us to confirm the identity of MBG in a woman plasma, before and during pregnancy.

These pioneering preliminary results will help to confirm previously similar results obtained on another MS device, leading to a promising perspective concerning the preeclampsia risk assessment. The method will be validated thanks to the accuracy profiles strategy.

At this time, a primary observational clinical study in pregnant women with non-pregnant controls is under design and will allow us to confirm previous results observed in pregnancy and in PE.

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