Marinobufagenin and its applications in the diagnosis of preeclampsia

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Introduction

- Marinobufagenin (MBG), an endogenous cardiotonic bufadienolide with vasoconstrictive activities, is a selective inhibitor of the α subunit of Na⁺K⁺-ATPase implicated in several pathophysiological circumstances that are characterized by hypertension and natriuresis, like in preeclampsia (PE).
- PE is a pregnancy-related disorder that consists in the development of hypertension and proteinuria after 20 weeks of gestation. Increased plasma MBG has been observed in mammals (rat and humans) presenting a preeclampsia syndrome[1-3], leading us to consider MBG as a biomarker for PE.
- This consideration implicates an accuracy and sensitive analytical method for MBG plasma levels quantification in order to further investigate the implications of MBG in PE. The final aim is to provide better comprehension of the phenomenon and potential new trends to diagnose the syndrome.

Extraction of pure MBG

- Parotid gland secretions of some toad species represent the main source of bufadienolides. Notably, MBG is the major cardiotonic steroid in the Bufo Marinus venom.
- Bufo Marinus is a toad species present in South America and introduced in Florida and Australia to control agricultural pests in sugar cane where it is currently becoming invasive.
- Given that no MBG standard is commercially available, we need to develop a successful extraction method to dispose of the reference compound. We made it from the crystallized form of the venom.

1) Preliminary step: Methanolic extraction and identification of MBG in Bufo Marinus venom

HPLC-UV (a) and MS/MS profile (b) of the principal spot at Rf 0.42

2) Quantitative extraction of pure MBG from Bufo Marinus venom by Flash Chromatography

Comparison of the two residues by TLC

Plasma extraction method

Solid Phase Extraction (SPE) process

The setup of the sensitive dosage method of MBG plasma levels started with an extraction from plasma samples by SPE. Several SPE sorbent phases were tested.

Conclusion and Outlooks

- We have developed a successful extraction method of MBG from Bufo Marinus crystallized venom and isolated pure MBG as a standard.
- A pre-extraction step from rat and human plasma has been carried out through SPE HLB (hydrophilic lipophilic balanced) cartridge with an extraction yield of 88%.
- Knowing that MBG plasma levels in preeclampsia are in the ng/ml range, several liquid chromatography strategies coupled with different detection methods allowing quantification of MBG in this range are considered.
- This dosage method once developed and validated will help to quantify MBG plasma levels of regular pregnant women and preeclamptic patients. We expect to be able to elucidate some biological questions such as: the biosynthetic origin of MBG and/or new routes for the diagnosis of the PE syndrome.

Acknowledgements: We thank the laboratory of Prof. Gerbeaux and Prof. Müller for their collaboration in the development of the extraction method to get pure MBG.