Marinobufagenin and its applications in the diagnosis of preeclampsia

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Introduction

- Marinobufagenin (MBG), an endogenous cardiotonic bufadienolide with vasocostrictive activities, is a selective inhibitor of the α1 subunit of Na⁺K⁺ATPase implicated in several pathophysiological circumstances that are characterized by hypertension and proteinuria, like in the preeclampsia syndrome (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.

Methods

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

- TLC of the methanolic extract of Bufo marinus venom (Mobile phase: Ethylacetate – Hexane – Ethylacetate – Relative by SiCj)
- HPLC- UV (a) and MS/MS profile (b) of the principal spot at RI 0.42

2) Quantitative extraction of MBG from Bufo Marinus venom

- A Flash Chromatography device was used to isolate a consequent amount of pure MBG
- Purification of the isolated Fractions

UPLC method development

1) Solid Phase Extraction (SPE) process

- The setup of the sensitive dosage method of MBG plasma levels starts with an extraction from plasma samples by SPE. Several SPE sorbent phases were tested. MBG concentration is assessed via UPLC-UV.

<table>
<thead>
<tr>
<th>Sorbent cartridge</th>
<th>MBG Peak area</th>
<th>Concentration (µg/ml)</th>
<th>Extraction yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPE HLB</td>
<td>300351</td>
<td>823,34</td>
<td>88,72%</td>
</tr>
<tr>
<td>SPE HLB optimized</td>
<td>256908</td>
<td>708,55</td>
<td>76,35%</td>
</tr>
<tr>
<td>SPE ACX</td>
<td>253491</td>
<td>692,08</td>
<td>74,57%</td>
</tr>
<tr>
<td>SPE WCI</td>
<td>235050</td>
<td>638,68</td>
<td>67,73%</td>
</tr>
<tr>
<td>SPE MAX</td>
<td>28514</td>
<td>778,34</td>
<td>83,87%</td>
</tr>
</tbody>
</table>

MBG presents the best extraction yield

2) UPLC characterization

<table>
<thead>
<tr>
<th>UPLC Conditions</th>
<th>Column</th>
<th>Gradient mobile phase</th>
<th>λ UV-detection</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BEN C18 1.7 µm</td>
<td>Water with 0.1% formic acid and ACN</td>
<td>296 nm</td>
<td>0.4 ml/min</td>
</tr>
</tbody>
</table>

MBG UPLC-Chromatogram

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, optimizations of the reversed-phase LC-UV method to allow quantifications of MBG in this range are currently under development.
- This dosage method once developed and validated will help to quantify MBG plasma levels of regular pregnant women and preeclamptic patients. By this, we will 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. Muller for their collaboration in the development of the extraction method to get pure MBG.