Rapid Electrochemical Simulation of the Oxidative Capsaicin Metabolism

Martin Eysberg[1]; Marco Kreidl[2]; Matthias Rainer[2]; Günther K. Bonn[2]; Herbert Oberacher[3]; Jean-Pierre Chervet[4]

[1]Antec Scientific, LLC, Boston, MA; [2]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innsbruck, Austria; [3]Institute of Legal Medicine and Core Facility Metabolomics, Medical University of Innsbruck, Innsbruck, Austria; [4]Antec Scientific, Alphen a/d Rijn, The Netherlands

Objective

Use of Electrochemistry–MS (EC-MS) for generation of Capsaicin metabolites.

- Purely instrumental approach
- Complementary to in vivo and in vitro techniques
- Access to phase I (oxidative) and phase II (adduct formation) metabolism
- Rapid and cost saving

1. Introduction

Capsaicin, known as the pungent ingredient in hot peppers, is rapidly metabolized in the human body by enzymatic processes altering the pharmacological as well as toxicological properties.
So far, 20 phase I metabolites and 23 GSH conjugates have been reported for capsaicin [1]. Due to its reactivity, more transformation products with pharmacological and toxicological relevance might exist.
The investigation of xenobiotic biotransformation based on in vivo and in vitro assays with laboratory animals, hepatic cells, or cell microsomes usually results in good agreement with the metabolism in the human body.
However, they are often costly, laborious, and time-consuming. Additionally, direct transferability of animal-based models is not always possible.
To overcome these limitations, instrumental approaches such as electrochemistry (EC) has been used [2].
In this study the oxidative transformation of capsaicin was investigated in vitro with electrochemistry as well as human liver microsomal (HLM) incubations [3].
The reaction mixtures were analyzed with LC-MS. Structure elucidation involved accurate mass measurements and multistage tandem mass spectrometry.

2. Schematics of this Study

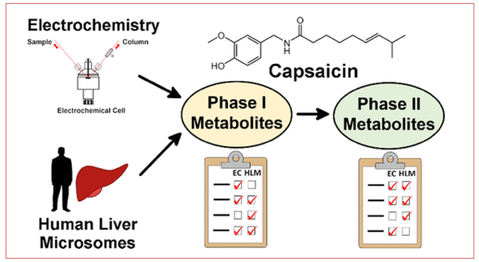
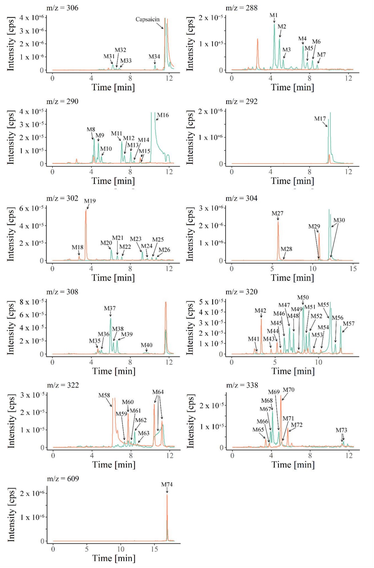


Figure 1: Electrochemically generated Capsaicin metabolites (phase I) were compared with metabolites generated by human liver microsomal incubations (EC vs. HLM). The reaction mixtures were analyzed with LC-MS. Phase II metabolites were generated by trapping the reactive intermediates with glutathione or with electrochemically activated ribonucleosides.
Evidence for the occurrence of some capsaicin metabolites in humans was obtained by urine screening.

3. Instrumentation

Electrochemical conversions were accomplished in an thin-layer cell (ReactorCell, Antec Scientific, The Netherlands). Potentials (E, 0–2000 mV) were applied using a ROXY™ Exceed potentiostat (Antec Scientific).
Sample solutions were pumped through the electrochemical cell at 5 µL/min. The effluent of the cell was directly connected to the injection valve of the LC system.
For the determination of massvoltammograms in data-dependent acquisition (DDA) mode and MSn experiments, an UltiMate 3000 LC system (Thermo Scientific) was hyphenated to a Finnigan LTQ linear ion trap (Thermo Scientific) with an ESI source. For the determination of exact masses, the LC system was hyphenated to a maxis Impact QqTOF-MS equipped with an ESI source (Bruker, Bremen, Germany).

4. Phase I Metabolites (EC vs. HLM)



ROXY™ Exceed Potentiostat equipped with electrochemical Reactor™Cell (Antec Scientific) for online EC-LC- MS

Use of boron dopped diamond (BBD) electrode

Figure 2: Comparison of extracted ion chromatograms obtained from LC-MS analysis of EC oxidation reactions at 2000 mV (turquoise) or HLM incubations (orange). Corresponding molecular structures were derived from accurate mass data (Δm ≤ 5 ppm) and MSn fragmentation patterns. Putative metabolite structures are outlined in Figure 3. Peaks that are not marked featured exact masses with Δm > 5 ppm.

5. Phase II Metabolites

Phase II metabolites were simulated by trapping reactive oxidation products with GSH or nucleosides. The sample solution was passed through the electrochemical cell at 2000 mV.
A solution of GSH (5 mM) in 10 mM ammonium formate (pH 7.4) and methanol (70/30, v/v) was added after the electrochemical cell via a T-piece at a flow rate of 5 µL/min.
The complete flow was pumped through a reaction tube before being collected in the sample loop.
For the generation of covalent nucleoside adducts, the sample solution contained 100 µM capsaicin and 175 µM guanosine, adenosine, or cytidine dissolved in 10 mM ammonium formate (pH 7.4) and methanol (70/30, v/v).

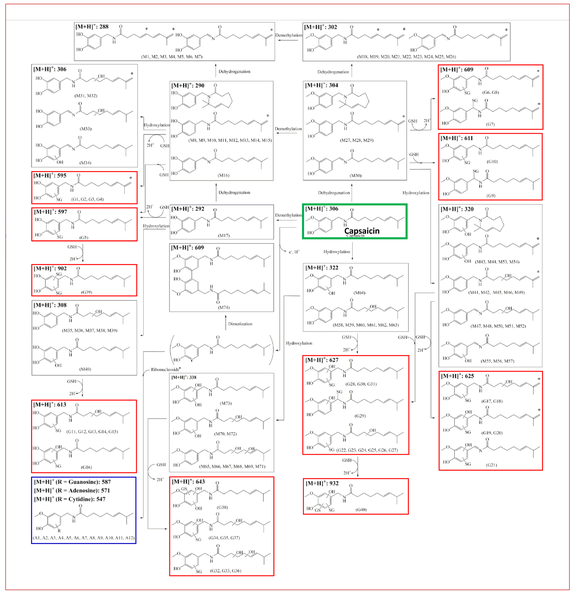


*Figure 3: Overview of putative structures of all metabolites generated in this study. Structures were derived from MS, MS2, and MSn data. Potential **phase I metabolites are encircled in black**, **phase II metabolites in red**, and **covalent adducts in blue**. Compounds, for which the exact localization of double bonds was not possible, are marked with asterisks (*).*

6. Phase I and II Metabolites Mimicked by EC and HLM

In Figure 4 a comparison is made for the metabolites generated by electrochemistry (EC) an human liver microsomal (HLM) incubations. The HLM results for both phase I and II are in good accordance with the EC results.

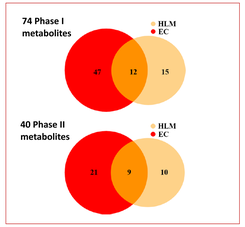


Figure 4: Comparison of metabolites mimicked by electrochemistry (EC) and by human liver microsomal (HML) incubations.
Of the 74 phase I metabolites generated by the two approaches, 20 have been already reported in the literature. 47 additional transformation products were generated by EC whereas 8 new products were detected after HLM.
For the 40 phase II metabolites, 16 of the detected conjugates have been previously reported in the literature. 21 additional GSH conjugates were detected after EC activation, while two new products were generated with both in vitro methods.

7. Conclusions

To gain new insights into potential biotransformation reactions of capsaicin, reaction mixtures obtained from Electrochemistry (EC) and HLM incubations were analyzed by LC–MS.
Both techniques have their advantages and limitations in predicting in vivo metabolisms. However, for capsaicin, the techniques complement each other.
Their combined use provided a comprehensive overview on transformation products occurring in vivo. With this dual approach, known oxidative pathways of capsaicin were reproduced. Furthermore, considerable number of additional transformation products were generated that have not been reported so far. Overall, the number of possible capsaicin oxidation products and conjugates was increased from 43 to 130.

- EC is an easy, purely instrumental approach
- EC is a very rapid technique (minutes) to generate and predict phase I and II metabolites
- EC complements HLM incubations w/o the need of isolation or extraction steps
- 47 new capsaicin transformation products were generated by EC, 8 new by HLM

EC and HLM incubations represent valuable tools to elucidate biotransformation pathways of important food ingredients and food additives.

References

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