Mimicking Drug Metabolism by EC/MS

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Introduction
For almost two decades electrochemistry (EC) has been successfully coupled to mass spectrometry. The electrochemical cell is used as a reactor in which a controlled oxidation or reduction takes place prior to MS detection. The oxidation products show excellent agreement with cytochrome P450 reaction products in nature (e.g., liver), mimicking the enzymatic biotransformation (biomimetic oxidation). This purely instrumental approach is making the use of costly enzymes and the risk of non-specific reactions obsolete. The reaction products are formed instantaneously in the EC cell allowing for direct infusion with MS and the measurement of short-lived compounds. Significant time and cost savings result using EC/MS compared to current in vitro (microsomes) or in vivo (rodents) approaches.

Methods / Instrumentation
All experiments were performed on ROXY EC system (Antec, The Netherlands) consisting of a Potentialstat, equipped with an electrochemical reactor cell and an infusion pump. A preparative electrochemical cell (µ-PrepCell, Antec, The Netherlands) equipped with a Glassy Carbon (GC) or Magic Diamond (BDD) working electrode were used in the experiments. For the oxidative fingerprint of the selected drug compounds, typically 10 µM solutions in 20mM ammonium formate/acetonitrile (50/50, v/v) were pumped through the electrochemical cell at 50 µL/min. Automated user defined programs were used to find the optimal potential. A Bruker HCT plus (Bruker Daltonics, Germany) mass spectrometer equipped with electrospray (ESI) source was used to monitor the oxidation products.

Mimicking Metabolism of Amodiaquine

For all drug compounds tested Amiodaquine, Verapamil and Norverapamil all electrochemically generated metabolites showed full agreement with the metabolites known from literature and/or from in-vitro and/or in-vivo experiments.

Conclusions
Using the ROXY™ EC system on-line with MS results in fast generation of metabolites in minutes vs. days or weeks using in-vitro and/or in-vivo methods. For all drug compounds tested Amiodaquine, Verapamil and Norverapamil all electrochemically generated metabolites showed full agreement with the metabolites known from literature and/or from in-vivo experiments.

The data demonstrate that hyphenation of EC with MS provides a powerful and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) on their oxidative metabolism. Furthermore, EC allows for fast synthesis of mg quantities of metabolites and becomes a truly "Metabolite Synthesizer".

References