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Untargeted screening of phase I metabolism of combretastatin A4 by multi-tool analysis

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ABSTRACT

The aim of the current study was to apply different strategies for generation of metabolites of combretastatin A4 (CA4) and subsequent identification of the unknown products of phase I metabolism. CA4 is a potent anti-tubulin agent currently undergoing clinical trials. The multi-tool analytical approach was based on electrochemistry (EC), *in silico* predictions, and *in vitro* studies with the use of rat liver microsomes. With the latter, two different analytical sample preparation methods were applied: protein precipitation and solid phase microextraction, both hyphenated to the liquid chromatography-high-resolution mass spectrometry platform (LC-HRMS). The EC was coupled directly to HRMS. Conventional techniques using enzyme fractions pooled from human or animals remain a method of choice for determinations of phase I of drug metabolism, EC and *in silico* methods, which enable determinations of metabolism patterns, are in turn considered to have great potential as fast alternatives to *in vitro* assays. While individual findings attained via employment of these four methods showed high similarity in relation to generated metabolic pathways for CA4, each method was found to provide unique features not identified with other approaches. In this paper, these differences are reviewed in view of potential artifacts and true metabolite production via various metabolism patterns under different experimental conditions. In addition, the reliability, applicability, MS compatibility issues, and potential of each of these technologies are discussed.

1. INTRODUCTION

Drug metabolism, as a biochemical process of transformation of pharmaceuticals within the organism, remains a main point of interest for clinicians, as comprehensive information regarding metabolic pathways and the mechanisms of action of new drugs is essential for recognition of their possible toxicity and biological activity in patients.

While drug metabolism is known to occur in different parts of human body, metabolic changes are mainly measured by study of liver microsomes, which contain specific enzyme systems. Drug metabolism is proceeded in reactions categorized into phase I and phase II. The main processes observed in the first phase include reduction, hydrolysis and oxidation. The latter applies to over 50% of all drugs and transformation occurs with participation of cytochrome P450 enzymes. The second phase, on the other hand, is mostly targeted at generally lipophilic compounds, and occurs through conjugation with highly polar endogenous compounds such as glucuronic acid, sulphate, and glutathione. Such compounds are modified through these reactions as hydrophilic components,