Evaluation of Dry Powder Preparations of Permeabilized *Pichia pastoris* Cells Expressing Human Cytochrome P450 2D6 Enzyme (CYP2D6) for Drug Metabolite Generation

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Overview

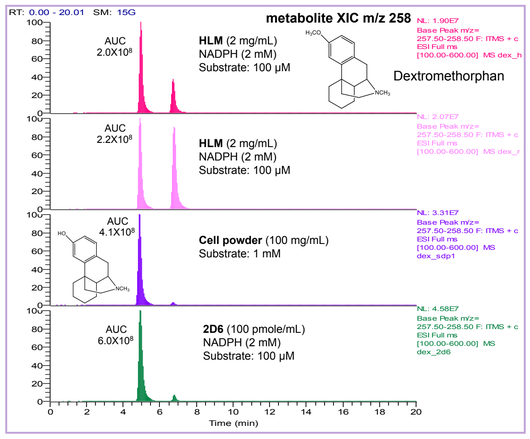
We present a new approach for generating drug metabolites by using dry powder preparations of permeabilized *Pichia pastoris* cells expressing the human Cytochrome P450 2D6 enzyme (CYP2D6). CYP2D6 substrates, amitriptyline, dextromethorphan, duloxetine, paroxetine, and thioridazine were all metabolized following incubation with the dry powder preparations in aqueous solutions. We also demonstrate that the dry powder preparations can be used in multiple reaction cycles exemplified by preparing the O-demethylation metabolite of dextromethorphan. The method represents a cost-effective alternative with scalability to obtain drug metabolites.

Introduction

The issuance of the FDA MIST guidelines has increased the need for monitoring drug metabolites in preclinical and clinical studies. Obtaining highly pure human drug metabolites by biotransformation plays an important role in drug discovery and development, for the purpose of determining chemical structures, serving as bioanalytical standards, or for testing biological and toxicological activities. Microsomal preparations of mammalian origin or membrane fractions from P450 enzyme over-expressed microbial or insect cells are often used to generate hard-to-synthesize metabolites. These can be cost-prohibitive for large-scale production of metabolites with low turnover rates. Here, we describe a new method using dry powder preparations of permeabilized *Pichia pastoris* cells expressing the human Cytochrome P450 2D6 enzyme (CYP2D6) for drug metabolite generation with scalability and potentially lower cost than conventional methods.

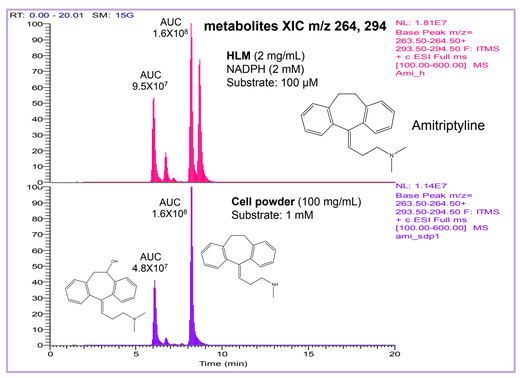
Methods

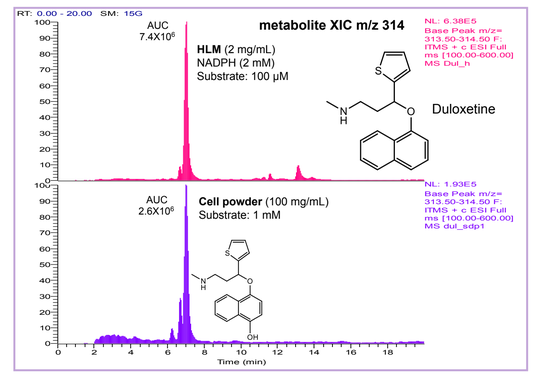
Pichia pastoris cells were metabolically engineered with the expression of human CYP2D6, NADPH-P450 oxidoreductase, and NADPH regenerating systems. Cell suspensions from fermentation at the stationary phase were dried transiently at a high temperature. For metabolite production, the powder was reconstituted and incubated in an aqueous solution with substrate addition. No NADPH was added. Human and rat liver microsomes, membrane fractions of CYP2D6 overexpressed microbial or insect cells were from commercial sources.



Results

CYP2D6 substrate metabolism at analytical scale by dry powder preparations, human/rat liver microsomes (H/RLM), and CYP2D6 membrane fractions from commercial sources (2D6), monitored using LC-MS





Scale-up preparation of the O-demethylation metabolite of Dextromethorphan using the dry powder preparations

Reaction cycles	Cofactor addition	Yield of metabolite (µM) (incubation with 500 µM substrate and 2 g cell powder in 20 mL)	Yield of metabolite (µM) (incubation with 1 mM substrate and 2 g cell powder in 20 mL)	Yield of metabolite (µM) (incubation with 500 µM substrate and 50 g cell powder in 500 mL)
I (4 hrs)	No	52 (supernatant)	62 (supernatant)	39 (supernatant)
II (4 hrs)	12 mM Glucose-6-phosphate	54 (supernatant)	78 (supernatant)	47 (supernatant)
III (overnight)	12 mM Glucose-6-phosphate	80 (cell suspension)	118 (cell suspension)	88 (cell suspension)
Total yield		186 (µM) (1.0 mg)	258 (µM) (1.3 mg)	174 (µM) (22.4 mg)

Discussion and Conclusions

Experimental results from our metabolism studies on CYP2D6 substrates with the dry powder preparations of *Pichia pastoris* cells indicated that the human CYP2D6 was functionally expressed in the yeast and remained active during the drying process at high temperatures, which made the cells more permeable. The dry powder preparations can also be used in multiple reaction cycles and permit large-scale preparation of drug metabolites with low turnover rates. With its relatively low cost, this method can potentially be a cost-effective alternative with scalability to obtain metabolites that are difficult or too costly to prepare using conventional methods.