

# Identification of a Functional Homolog of the Mammalian CYP3A4 in Locusts

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Received January 31, 2014; accepted April 28, 2014

## ABSTRACT

Insects have been proposed as a new tool in early drug development. It was recently demonstrated that locusts have an efflux transporter localized in the blood-brain barrier (BBB) that is functionally similar to the mammalian P-glycoprotein efflux transporter. Two insect BBB models have been put forward, an *ex vivo* model and an *in vivo* model. To use the *in vivo* model it is necessary to fully characterize the locust as an entire organism with regards to metabolic pathways and excretion rate. In the present study, we have characterized the locust metabolism of terfenadine, a compound that in humans is specific to the cytochrome P450 enzyme 3A4. Using high-resolution mass spectrometry coupled to ultra-high-performance liquid chromatography, we have detected

metabolites identical to human metabolites of terfenadine. The formation of human metabolites in locusts was inhibited by ketoconazole, a mammalian CYP3A4 inhibitor, suggesting that the enzyme responsible for the human metabolite formation in locusts is functionally similar to human CYP3A4. Besides the human metabolites of terfenadine, additional metabolites were formed in locusts. These were tentatively identified as phosphate and glucose conjugates. In conclusion, not only may locusts be a model useful for determining BBB permeation, but possibly insects could be used in metabolism investigation. However, extensive characterization of the insect model is necessary to determine its applicability.

## Introduction

Today, most new drugs that fail in the clinical phase do so due to lack of desired pharmacological activity or toxicity (Hughes et al., 2011). In the development of central nervous system drugs, the blood-brain barrier (BBB) creates additional challenges, and therapeutic agents for the treatment of neurologic disorders often fail in clinical trials due to inadequate BBB permeation (Alavijeh et al., 2005; Geldenhuys et al., 2012). *In vitro* methods for testing BBB penetration are too simple, not integrating the complexity of the BBB, and therefore may lack important functions of the BBB (Naik and Cucullo, 2012). Preclinical *in vivo* models are very costly in terms of time and use of animals (rodents). Fast screening models for BBB permeation are therefore very attractive.

Insects have been suggested as novel models in drug discovery (Geldenhuys et al., 2012). It has been demonstrated that locusts, among other insects, have an efflux transporter that is functionally similar to the mammalian P-glycoprotein efflux transporter (Nielsen et al., 2011).

Currently, two insect BBB models have been reported using locusts as model insects (Nielsen et al., 2011; Andersson et al., 2013). An *ex vivo* model has been developed and allows application of test items directly to entire brains isolated prior to testing. Besides this model, an

*in vivo* model has also been developed in which the test item is injected in the hemolymph and may need to pass the barriers of metabolism and excretion before reaching the brain. As such, the *in vivo* locust model may have more similarities to other *in vivo* models like rodent models. Also, the brain remains in its natural environment, with no risk of impairing the barrier function.

Despite obvious anatomic differences, there are many physiologic and biochemical similarities between insects and mammals. Essential systems such as protein synthesis and cell metabolism are not significantly different between insects and mammals (Klowden, 2007). Monooxygenases like the cytochrome P450 (P450) enzymes are found in virtually all eukaryotic organisms, including insects (Scott, 2008). This is of specific interest as some of the most important drug-metabolizing enzymes in humans are the P450 enzymes. Among mammalian P450 enzymes, the subfamilies CYP1A1/2, CYP2B6, CYP2C9/19, CYP2D6, CYP2E1, and CYP3A4/5 are the main contributors, and CYP3A4 accounts for the metabolism of almost half of all marketed drugs (Guengerich, 2008).

Most literature on insect metabolic systems concerns insecticide detoxification mechanisms and resistance development (Smith, 1962; Wilkinson and Brattsten, 1972; Feyereisen, 1999; Scott and Wen, 2001). Furthermore, most research was conducted in the 1950s and 1960s when the analytic methods were limited compared with the technologies available today.

In the present study, we have characterized the metabolism in locusts of the antihistaminic drug terfenadine, a specific human CYP3A4 substrate.

This work was supported by the Danish National Advanced Technology Foundation [Grant 023-2011-3].  
dx.doi.org/10.1124/dmd.114.057430.

**ABBREVIATIONS:** AUC, area under the curve; BBB, blood-brain barrier; DMSO, dimethylsulfoxide; HLM, human liver microsome; MS, mass spectrometry; MS<sup>2</sup>, tandem mass spectrometry; P450, cytochrome P450.