Effective Inhibition of CYP3A4-Dependent Drug Oxidation by Anti-CYP3A4 Antibodies in MetMax™ Cryopreserved Human Hepatocytes

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INTRODUCTION

• The development of new therapeutics requires precise identification of the enzyme(s) underlying their initial metabolism (reaction phenotyping).
• Reaction phenotyping studies are routinely performed with human liver microsomes (HLM) paired with P450 enzyme-specific chemical or antibody inhibitors to identify the relative contribution (fm) of individual P450s in the metabolism of a specific drug or drug candidate.
• Human hepatocytes are considered the “Gold Standard” for in vitro studies of hepatic drug metabolism due to the presence of all liver drug-metabolizing pathways, thereby providing a better estimation of fm.
• Human hepatocytes coupled with inhibitory P450 antibodies represent an ideal experimental system for reaction phenotyping. However, this approach cannot be used with intact hepatocytes due to the inability of antibodies to enter the cells.
• Coupling hepatocytes with inhibitory antibodies for reaction phenotyping is, however, possible with MetMax™ human hepatocytes due to their permeabilized plasma membranes.
• We report here on the success of this novel approach

OBJECTIVES

To validate a unique and robust approach to P450 reaction phenotyping, namely one utilizing specific, inhibitory P450 antibodies together with MetMax™ cryopreserved human hepatocytes which, due to their altered permeability, enable the exogenous antibodies to reach their intracellular target antigens.

SUMMARY

CYP Immunoinhibit Kit P450 antibodies are capable of eliciting extensive inhibitory effect on drug metabolism by their cognate enzymes in MetMax™ cryopreserved human hepatocytes.

• Dose-dependent inhibition of CYP3A4-catalyzed MDZ hydroxylation was observed in MetMax™ cryopreserved human hepatocytes by native anti-CYP3A4 IgG
• Analogous dose-dependent decreases in MetMax™-mediated substrate metabolism were noted with the five other human P450 antibodies tested

The results indicate that the permeabilization process used to derive MetMax™ hepatocytes enables passage of large IgG molecules through the plasma membranes of these cells.

Since assay conditions used with MetMax™ hepatocytes and CYP Immunoinhibit Kit antibodies employ saturating substrate concentrations, the maximum metabolic contribution of each target P450 can be determined. The results obtained can be easily interpreted and do not require extrapolation (e.g., ISEF or RAF) to assess the contribution of a given P450 enzyme to lead compound metabolism.

Importantly, P450 reaction phenotyping has:
• a) facilitated the appropriate structural modifications of experimental therapeutics in order to improve their metabolic stability;
• b) permitted estimates of the potential for drug-drug interactions
• c) allowed assessment of species and interindividual differences in oxidative metabolism.
Thus, a more accurate reaction phenotyping process, such as that described here, should allow for the identification of drug candidates with less liabilities stemming from their metabolism.

REFERENCES