Induction of drug metabolizing enzymes is one critical component in conferring drug-drug interactions. The process is controlled through use of biotransforming enzymes in cells, leading to reactions which are regulated gene transcription. One mechanism is the control of the expression of these enzymes through induction by drugs. There is a critical need for the upregulation of phase I and phase II enzymes such as cytochrome P450 (CYP) enzymes, which play a key role in drug metabolism. The regulation of activity of CYP1A1 in hepatocytes using phenacetin as a substrate exist under regulatory conditions. The identification and quantification of the substrates is key for the elucidation of the mechanisms of drug metabolism. The P450-dependent metabolism of xenobiotics requires biochemical methods for determination. The P450-Glo Assay with luciferin-1A2 is a sensitive and effective method of determining CYP1A2 induction in Hepatocytes, which will utilize data expeditiously and simplify workflow. Herein, we describe the development of a protocol for measuring CYP1A2 induction in Hepatocytes. The protocol will utilize luciferin-1A2 as the substrate and phenacetin as the inducer. The induction rates were similar to the inducers for the 1A family with the majority by CYP1A2. CYP2C19 and CYP3A4 were found to be a specific substrate for CYP3A4 and able to be used as a marker for the metabolism of phenacetin. The study demonstrates luciferin-1A2 offers a fast, reliable and low-cost alternative to the current gold standard of assessing drug-drug interactions.

MATERIALS & METHODS

Luciferin-1A2 Substrate

Luciferin-1A2, a derivative of 7-hydroxymethyl coumarin, is a specific substrate for CYP1A2, CYP1B1, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP5A1, CYP6A2, CYP7A1, CYP8B1, CYP9A1, CYP11B2, CYP17A1, and aldehyde dehydrogenase 1 (ALDH1). Luciferin-1A2 is a highly sensitive substrate for CYP1A2 with a detection limit of 0.01 nM. The reaction of 5 nM Luciferin-1A2 with 50 µM omeprazole (PC) or 1% acetonitrile (VC) for 48 hours prior to metabolism with CYP1A2 substrates. The fold inductions were compared between the substrates Luciferin-1A2 and phenacetin. Panel A. represents the data derived by substrate specificities with recombinant P450 enzymes. Specificity of Luciferin-1A2 and phenacetin was measured for each CYP enzyme using the P450-Glo Assay with Luciferin-1A2. Luciferin-1A2 was primarily metabolized by the CYP1 family. Notable substrate specificity was observed with CYP1A2 and CYP1B1. The study demonstrates Luciferin-1A2 offers a fast, reliable and low-cost alternative to the current gold standard of assessing drug-drug interactions.

Comparison of luciferin-1A2 andphenacetin

Luciferin-1A2 is a specific substrate for P450 1A family and comparable to phenacetin. Different substrates were observed and should be taken into account depending on the application. Lytic method compared more favorably than the non-lytic method for the application of determining induction of CYP1A2. Luciferin-1A2 was used in conjunction with cryoplateable human hepatocytes offers a fast, reliable and low-cost alternative to the current gold standard of assessing induction potential of new chemical entities with substrates requiring traditional bioanalytical methods.

Conclusion

Luciferin-1A2 is a specific substrate for P450 1A family and comparable to phenacetin. Different substrates were observed and should be taken into account depending on the application. Lytic method compared more favorably than the non-lytic method for the application of determining induction of CYP1A2. Luciferin-1A2 was used in conjunction with cryoplateable human hepatocytes offers a fast, reliable and low-cost alternative to the current gold standard of assessing induction potential of new chemical entities with substrates requiring traditional bioanalytical methods.