Validation Of An In-Vitro Canine Primary Hepatocyte-Based Co-Culture System
For Use In Extended DMPK/Tox Studies

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ABSTRACT

Accurately predicting the pharmacokinetic and toxicological properties of drugs is essential in developing new medicines. The need for streamlined and cost-effective systems that can accurately predict human response is critical. The objective of this study was to develop and validate a canine co-culture model that can perform the same function as in vivo studies, which is critical for drug development. The model was designed to classify compounds into three categories: hepatotoxic, non-hepatotoxic, and intermediate. The model was tested on a set of 31 compounds, including 11 reference low clearing compounds. In other studies, toxicity of 51 compounds was evaluated. These results indicate that this canine co-culture system outperforms two other widely used cellular systems, and represents a novel and effective platform for predicting pharmacokinetics and toxicity profiles of drugs under development.

MATERIALS AND METHODS

All compounds and reagents were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, USA) except bromfenac, micropore, telaprevir, zileuton, tergriazine, rivaroxaban and carbamazepine which were obtained from Sequoia (Parsippany, NJ, USA). Dog co-culture model: pre-expired dog hepatocytes were purchased from Charles River (Wilmington, Massachusetts). The co-culture models were a mixture of dog hepatocytes and non-parenchymal stromal cells (proprietary information). All dog co-culture models were plated on collagen coated plates (proprietary ratios).

Compound selection and concentrations used: the compounds tested included 51 reference compounds (40 drugs known to be hepatotoxic in humans and 11 which are not known to be hepatotoxic in humans) according to the liver toxicity knowledge data base (LTKB) (Chen et al., 2011) and literature data in absence of data in the LTKB. The concentrations used were not fixed, but were selected based on Cmax values found in literature as well as in commercial databases. Cmax refers to the maximal concentration of a given compound in human blood. The concentrations tested were multiple of Cmax, i.e., 12.5, 25, 50, 75, 90, and 100 Cmax for the HepG2 and the primary human hepatocytes. For the dog co-culture model, the same concentrations were tested including two extra concentrations (i.e., 5 and 2.5 Cmax). The concentrations of the metabolites were monitored over one hour in culture and normalized to day 1 or withdrawn, dark yellow (DILI score of 4 and 5), light yellow (DILI score between 1 and 3), white (no DILI score) and black (no information).

CONCLUSIONS

The model was designed to classify compounds into three categories: hepatotoxic, non-hepatotoxic, and intermediate. The model was tested on a set of 31 compounds, including 11 reference low clearing compounds. In other studies, toxicity of 51 compounds was evaluated. These results indicate that this canine co-culture system outperforms two other widely used cellular systems, and represents a novel and effective platform for predicting pharmacokinetics and toxicity profiles of drugs under development.