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Appraisal of state-of-the-art

Investigation of the utility of published in vitro intrinsic clearance data for prediction of in vivo clearance

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Abstract

Introduction: This study was conducted to compare and contrast published in vitro intrinsic clearance values reported for compounds from different laboratories and the predictivity of these data to project in vivo clearance. **Methods:** A total of 103 compounds were selected for investigation and an exhaustive literature search was conducted to identify in vitro intrinsic clearance (CL,i) values for comparative purposes. The simple well-stirred model was used to predict in vivo clearance using these in vitro intrinsic clearance values. **Results:** Data were available in the literature for <10% of the compounds of interest in rat, dog, monkey, or human S9, as well as <10% for dog or monkey microsomes or hepatocytes. Therefore, this comparative exercise was limited to rat and human microsomes and hepatocytes. Examination of the available CL,i values indicated a substantial (up to 100 s-fold) variation in values reported in the literature; this variability translated into substantial variation in predicted in vivo clearance. **Discussion:** The literature paucity and variability described here demonstrate the importance of generating experimentally consistent de novo CL,i data for the purpose of method validation or in vitro–in vivo scaling.

Keywords: Hepatocytes; In vivo; In vitro; Intrinsic clearance; Microsomes

1. Introduction

The reduction of attrition during drug development is one of the major financial and strategic challenges within the pharmaceutical industry (Kola & Landis, 2004). One key area of pharmaceutical attrition can be attributed to drug metabolism and pharmacokinetic (DMPK) properties. Although increased attention to and investment in this area have substantially reduced DMPK-related attrition over the past decade (Frank & Hargreaves, 2003), continued improvement in the a priori prediction of human pharmacokinetic parameters remains of substantial interest.

One DMPK-related tool increasingly employed by pharmaceutical scientists during drug discovery is in vitro metabolic turnover, or intrinsic clearance (CL,i). CL,i assays are reasonably facile to perform and can be automated for higher-throughput screening (Ansede & Thakker, 2004; Di et al., 2003; Eddershaw & Dickins, 1999; Janiszewski et al., 2001). The CL,i determination can be generated in several different matrices (microsomes, S9, hepatocytes), and data are derived from a wide variety of experimental conditions. However, despite their growing popularity and use in drug discovery, caution has also been advised in the use of in vitro CL,i determinations for the prediction of in vivo clearance, in that inaccurate in vivo predictions are frequently yielded (Andersson, Bredberg, Ericsson, & Sjoberg, 2004; Clarke & Jeffrey, 2001).

As part of an ongoing exercise in this laboratory in the area of interspecies extrapolation of pharmacokinetic parameters, the ability of preclinical in vivo pharmacokinetic data from rat, dog, and monkey to predict human clearance has recently been explored (Ward & Smith, 2004). However, this assessment did not evaluate the predictive ability of in

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vitro data, alone or in combination with preclinical animal data, to project human clearance as has been previously proposed (Lave, Coassolo, & Reigner, 1999; Obach, 1999; Obach et al., 1997). Indeed, the lack of inclusion of in vitro intrinsic clearance for the various molecules in the in vivo scaling exercise has been a key point of concern at various scientific venues where these findings have been presented, and there is a general presumption that such data are both available in the literature and of sufficient quality to incorporate in extrapolation. Additionally, numerous laboratories have been publishing in vivo – in vitro modeling exercises based on literature in vitro intrinsic clearance data for various molecules, but with little work being done to investigate or explain any observed differences in such values for given molecules (Houston, 1994; Houston & Carlile, 1997; Lave et al., 1997). Therefore, the objective of the present investigation was to comprehensively survey the available literature for the 103 molecules employed in the initial in vivo extrapolation exercise to identify existing in vitro microsomal, S9, and/or hepatocyte CL,i data for rat, dog, monkey, and/or human. Attempts were then made based on the identified data to compare values reported across studies, and to extrapolate these values to in vivo clearance.

2. Materials and methods

The compounds studied in this investigation were those 103 compounds described previously for which in vivo rat, dog, monkey, and human clearance data were available (Ward & Smith, 2004). In vitro preparations or models using microsomes, hepatocytes and/or S-9 fraction are routinely employed in the pharmaceutical industry to study the metabolism and fate of the xenobiotics and are the models of choice for estimation of intrinsic clearance. Each matrix has various attributes and detriments for the potential prediction of in vivo clearance, including differing qualitative and quantitative content of drug metabolizing enzymes and other proteins, preparation metabolic activity, and viability. The utility of CL,i in the prediction of in vivo clearance has been proposed by several authors (Griffin and Houston, 2004; Houston, 1994; Lave et al., 1997; Obach, 2001; Obach et al., 1997). An exhaustive review of the literature was performed to seek corresponding in vitro CL,i data for these 103 compounds in microsomes, S9 fraction, or hepatocytes, from rat, dog, monkey, or human. The literature search was conducted using Medline spanning the years from 1966 to present. The terms used as keywords to assist in the search included “intrinsic clearance”, “in vitro”, “compound name”, “microsomes or microsomal”, “S-9 or S9”, “hepatocytes” and/or a combination of any of the search terms. Likewise, literature reviews and compilation articles containing metabolic stability data also were searched for the target compounds. Similarly, a Medline search was also performed to identify corresponding human plasma protein binding values for the compounds in the dataset for scaling purposes.

For those articles wherein metabolite formation, rather than substrate disappearance data, were reported, the CL,i was determined using the following equation:

$$\mathrm{CL,i} = \sum_{j=1}^{n} \frac{V^{j}_{\max}}{K^{j}_{\mathrm{m}}}$$

where Vmax is the maximal velocity of the metabolic reaction, Km is the Michaels–Menten constant, and j represents the number of metabolites quantified in the study.

For studies which reported substrate depletion data, the CL,i was calculated by dividing the first-order elimination rate constant by the microsomal or hepatocyte concentration of the incubations, and expressed in units of μl/min/mg protein or μl/min/10^6 cells. Where necessary, conversion factors of 10 g liver/standard rat weight and 250 g standard rat weight were used (Iwatsubo et al., 1997).

Finally for comparison purposes, the in vivo clearance of the compounds in the dataset was predicted from the in vitro data using the simple well-stirred model, given below (Pang & Rowland, 1977):

$$\mathrm{CL_{in\ vivo}} = \frac{\mathrm{fu}\cdot\mathrm{CL,i}\cdot Q_{\mathrm{H}}}{\mathrm{fu}\cdot\mathrm{CL,i} + Q_{\mathrm{H}}}$$

where fu=fraction unbound in plasma, Q_{H}=hepatic blood flow, and Q_{H} values of 85 and 21 ml/min/kg (Davies & Morris, 1990; Uhing et al., 2004) were used for rat and human, respectively. For this scaling calculation, the CL,i values were converted into ml/min/kg using the scaling factors described earlier (Iwatsubo et al., 1997). For this scaling exercise, clearance was predicted both with and without plasma protein binding correction. The literature search yielded plasma protein binding values for most of the compounds in human plasma, however, the percentage of compounds for which plasma protein binding values in rat were available was less than 5%, therefore, no protein binding correction factor was used in the prediction of rat in vivo clearance from the identified CL,i values. With respect to the actual predictivity of the various matrices or values used, this exercise was not intended to be an exhaustive exploration, and therefore did not include such considerations as tissue protein binding, blood cell association, or the use of different scaling models. For comparison of the accuracy of different methods used in this exercise, the mean absolute error (MAE) was estimated as:

$$\mathrm{MAE} = \frac{\sum_{i=1}^{n} \left|\mathrm{CL}^{i}_{\mathrm{predicted}} - \mathrm{CL}^{i}_{\mathrm{observed}}\right|}{N}$$

where i represents the individual observation, N=the total number of observations in the set, $\mathrm{CL_{predicted}}$=clearance predicted using the average of the published CL,i data and $\mathrm{CL_{observed}}$=observed in vivo clearance.

3. Results

An exhaustive search of the available literature was conducted to seek in vitro metabolic turnover data from rat, dog, monkey and/or human in hepatic microsomes, S9, or