Prediction of clearance from the HepaRG cell system: comparison with human hepatocytes for a range of substrates

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Introduction

Predictions of in vivo intrinsic clearance (Cl_{int}) from cryopreserved human hepatocytes may vary considerably from donor to donor and pooling has been used to reduce this effect.
HepaRG have been proposed as an alternative complete model to evaluate hepatotoxicity with potential as a quantitative model for prediction of metabolic clearance. The HepaRG cell line is derived from a liver tumor of a female patient suffering from hepatocarcinoma and after appropriate culture procedures differentiated in hepatocyte-like and biliary-like cells, in a ratio of 1:1 expressing a relevant set of drug metabolism enzimes, transporters and hepatobiliary markers (1).
In this work, we compare the intrinsic clearance obtained for a set of 40 well known drugs using a donor pool of human cryopreserved hepatocytes with those obtained with the hepatoma-derived cell line HepaRG.

Materials & Methods

Differentiated cryopreserved HepaRG cells from Biopredic Int. and pooled human cryopreserved hepatocytes from CellzDirect (250,000/ml) were used.
Disappearance of the compounds (1µM) was followed up to 45min and Cl_{int} derived by dividing the fitted constant K in eq.1 by the concentration of cells, using the hepatocellularity parameters reported (2,3) and estimating the non-specific binding eq. 2 (4):

eq1: $C_t = C_0 \cdot e^{-k_e \cdot t}$

eq.2: $fu_{hepz} = \frac{1}{1 + 125 \cdot V_R \cdot 10^{0.072 log\frac{P}{D^2} + 0.067 log\frac{P}{D} - 1.126}}$

Cl_{int} was deduced by Cl in vivo according to Well Stirred model (WS) eq.3 or Parallel Tube (PT) eq. 4 models:

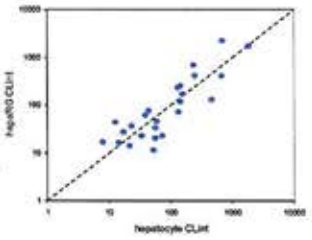
eq.3: $Cl_{int} = \frac{CL_b}{\frac{fu_p}{R_b} \cdot \left(1 - \frac{CL_b}{Q_H}\right)}$

eq4: $CL_{int} = -ln\left[\frac{Q_H - CL_b}{Q_H}\right] \cdot \frac{Q_H}{fu_p / R_b}$

The accuracy was measured by average fold error (AFE) eq. 5: $afe = 10^{\frac{1}{n}\sum log\frac{predicted}{observed}}$

Results

•CL_{int} measured from HepaRG cells closely correlated with Clint measured with cryopreserved hepatocytes (Figure 1).



•Prediction of Clearance with both Hepatocytes (Fig. 2A) and HepaRG (Fig. 2B) was very variable, similar to previous experience (5).

• No significant amelioration was achieved using PT instead of WS

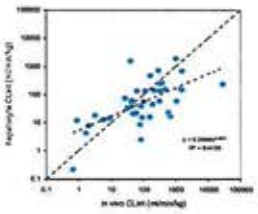


Fig. 2A:
Comparison of Clint predicted from cyopreserved Human hepatocytes and Clint in-vivo

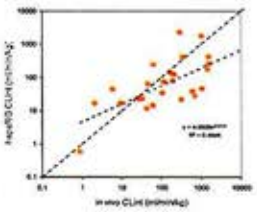


Fig.2B:
Comparison of Clint predicted from HepaRG cells and Clint in-vivo

• For both cryopreserved hepatocytes and HepaRG, on average, the cells underpredicted unbound Cl_{int} derived from in vivo plasma clearance, with marginal differences in AFE being dependent on the liver model used: 1.9 and 1.4 for the well stirred (WS) and parallel tube (PT) respectively, in cryopreserved hepatocytes; and 2.7 and 1.8 for the WS and PT model respectively, in HepaRG.

•In both systems, AFE was dependent on clearance, with increasing under-prediction observed with increasing clearance.

	AFE			
Clint in-vivo (WS)	<10 ml/min/kg	10-100 ml/min/kg	100-1000 ml/min/kg	>1000 ml/min/kg
Cryopreserved Pooled Hepatocytes	0.4	1.4	2.7	8.5
HepaRG	1.6	1.4	2.8	7.2

•Greater under-prediction was observed with increasing protein binding irrespective of liver model (Figure 3).

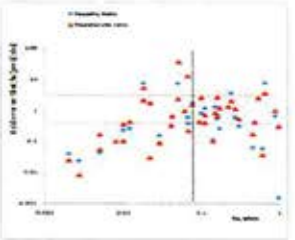


Fig.3:
fold errors as function of Protein binding.
3 folds limit are shown

• Limitations to in-vitro Clearance are not due to the permeability of compounds to hepatocytes, since apparent permeability, as assessed by PAMPA, did not correlate with the fold errors of Fig. 3 (data not shown). This suggests that the main difference between vivo and vitro in terms of permeability may be due to transporters activity.

Conclusion

•A novel system for prediction of clearance, the HepaRG cell, has been shown for the first time to offer drug metabolising enzyme activity at the same level as pooled human hepatocytes which would enable quantitatively similar results.

•Both the HepaRG and hepatocyte systems show clearance dependent prediction bias which appears to be related to protein binding.

•Possible improvements to prediction methodology for hepatocytes, such as empirical correction of bias, could be equally applied to the HepaRG system.

References

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