In Vitro Stability of Human Recombinant Cytochrome P450 Enzymes

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INTRODUCTION

Human recombinant cytochrome P450 enzymes (rCYPs) are used extensively in pre-clinical drug development because of their ready availability and compatibility with high throughput drug metabolism studies. In most *in vitro* drug metabolism studies an excess amount of parent compound is used and metabolite formation rate is monitored. Linearity of the latter with time is assumed when calculating kinetic parameters. The higher concentration of parent compound relative to metabolites obviates the possibility of product inhibition, and hence minimises non-linearity of metabolite formation with time. However, linearity is often assessed based on regression analysis on very few samples, assuming that the enzyme is stable throughout incubation. The purpose of this study was to investigate *in vitro* enzyme stability.

METHODS

Gentest (www.gentest.com) and Cypex (www.cypex.co.uk) provide comprehensive data on the time-course of product formation in rCYP systems with multiple samples. Using these data the stability of different rCYPs with time was examined indirectly. The data were fitted using WinNonLin (V4.0, Pharsight) by a model incorporating the classical Michaelis-Menton equation without or with the assumption of enzyme stability (Eqs. 1-3). For the latter, enzyme degradation was assumed to follow first-order kinetics.

$$dM_t / dt = V_{max} S_t E_t / (K_m + S_t) \quad (1)$$

$$dS_t / dt = -dM_t / dt \quad (2)$$

$$dE_t / dt = -k_{deg} E_t \quad (3)$$

where M_t, S_t, E_t are the formed metabolite concentration, substrate concentration , and active enzyme abundance at time t, respectively; V_{max} is the maximum rate of metabolism; K_m is the Michaelis constant; and k_{deg} is the first-order degradation constant.

RESULTS

Assuming first-order enzyme degradation improved the fit for all data sets (based on the Akaike Information Criterion) with one exception (Figs. 1&2), where the enzyme was stable throughout the study period (60 min, see Fig. 1g). The median value of $t_{0.9}$ (time associated with maintenance of 90% enzyme activity) was 5.9 min, and estimates of half-lives ($t_{0.5}$) for apparent decline in activity ranged from 11 to 231 min (Table 1).

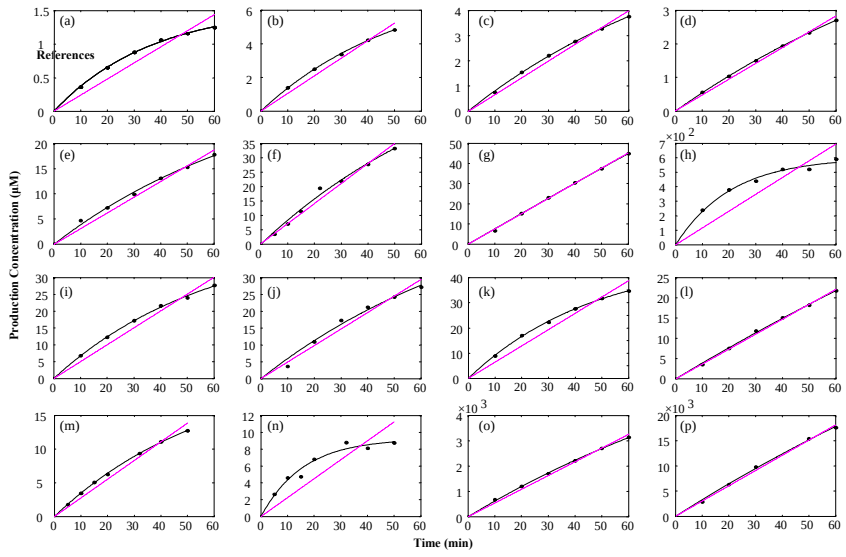

Fig. 1 Time course of product formation in Gentest rCYP systems, fitted by models without (dotted line) or with (solid line) the assumption of enzyme stability. (a) CYP1A2 + Reductase, (b) CYP2A6 + Reductase, (c) CYP2A6 + Reductase + b5, (d) CYP2B6 + Reductase, (e) CYP2C8 + Reductase, (f) CYP2C9*1 + Reductase, (g) CYP2C9*1 + Reductase + b5, (h) CYP2C18 + Reductase, (i) CYP2C19 + Reductase, (j) CYP2C19 + Reductase + b5, (k) CYP2D6*1 + Reductase, (l) CYP2E1 + Reductase + b5, (m) CYP3A4 + Reductase, (n) CYP3A4 + Reductase + b5, (o) CYP3A5 + Reductase, (p) CYP3A5 + Reductase + b5

CONCLUSIONS

The results suggest that typical time-linearity studies, with very few samples, may not allow enzyme instability to be identified leading, potentially, to inaccurate characterisation of metabolite formation rates and apparent atypical kinetics.

Table 1. *In vitro* stability of human recombinant cytochrome p450 enzymes

Source	Human recombinant p450	k_{deg} (min^{-1})	$t_{0.5}$ (min)	$t_{0.9}$ (min)
Gengest	CYP1A2 + Reductase	0.027±0.001	25.7	3.9
	CYP2A6 + Reductase	0.019±0.001	36.5	5.5
	CYP2A6 + Reductase + b5	0.011±0.001	63.0	9.6
	CYP2B6 + Reductase	0.008±0.000	86.6	13.2
	CYP2C8 + Reductase	0.012±0.002	57.8	8.8
	CYP2C9*1 + Reductase	0.012±0.004	57.8	8.8
	CYP2C9*1 + Reductase + b5	0.0007±0.0010 §	-	-
	CYP2C18 + Reductase	0.048±0.005	14.4	2.2
	CYP2C19 + Reductase	0.017±0.001	40.8	6.2
	CYP2C19 + Reductase + b5	0.011±0.005	63	9.6
	CYP2D6*1 + Reductase	0.021±0.001	33	5.0
	CYP2E1 + Reductase + b5	0.003±0.001	231	35.1
	CYP3A4 + Reductase	0.017±0.001	40.8	6.2
	CYP3A4 + Reductase + b5	0.063±0.011	11.0	1.7
	CYP3A5 + Reductase	0.007±0.001	99.0	15.1
	CYP3A5 + Reductase + b5	0.0045±0.0019	154	23.4
Cypex	CYP1A2R Bactosomes	0.019±0.004	36.0	5.5
	CYP2A6R Bactosomes	0.050±0.002	14.0	2.1
	CYP2B6R Bactosomes	0.051±0.003	13.6	2.1
	CYP2C8R Bactosomes	0.016±0.002	43.4	6.6
	CYP2C18R Bactosomes	0.059±0.004	11.7	1.8
	CYP2C19R Bactosomes	0.023±0.002	30.5	4.6
	CYP2D6R Bactosomes	0.030±0.004	22.8	3.5
	CYP2E1R Bactosomes	0.035±0.001	20.0	3.0
	CYP3A4R Bactosomes	0.044±0.002	15.7	2.4
	CYP3A5LR Bactosomes	0.007±0.001	96.2	15.1
Median		0.018	38.6	5.9

§ Enzyme was stable during the incubation time (60 min)

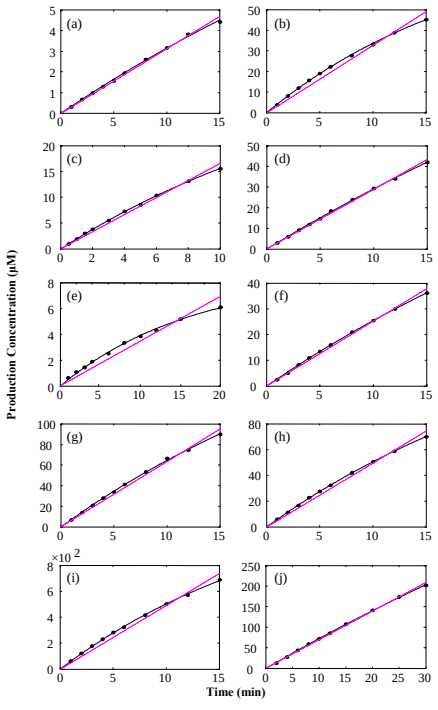

Fig. 2 Time course of product formation in Cypex rCYP systems, fitted by models without (dotted line) or with (solid line) the assumption of enzyme stability. (a) CYP1A2R Bactosomes, (b) CYP2A6R Bactosomes, (c) CYP2B6R Bactosomes, (d) CYP2C8R Bactosomes, (e) CYP2C18R Bactosomes, (f) CYP2C19R Bactosomes, (g) CYP2D6R Bactosomes, (h) CYP2E1R Bactosomes, (i) CYP3A4R Bactosomes, (j) CYP3A5LR Bactosomes