Cytochrome P450 Turnover:
Methods for Determining Rates and Implications for the Prediction of Drug Interactions

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Introduction

The molecular and cellular regulation of cytochrome P450 (CYP) enzymes is a complex process involving a network of transcription factors interacting with multiple promoter/enhancer elements, and various mechanisms of stabilisation and degradation [1]. At a kinetic level, it is generally accepted that *de novo* enzyme synthesis may be described as a (pseudo-) zero-order process, and enzyme degradation as a first-order process. Various *in vitro* and indirect *in vivo* approaches have been used to estimate the turnover half-lives of CYPs. We will summarise each of these methods, emphasising the lack of current consensus on values for specific CYPs, and the implications of this in predicting the extent and time- course of drug interactions involving induction and mechanism (time)-based inhibition (MBI).

Regulation of CYP Enzymes

The expression of CYPs might be affected by a number of influences involving physiological (hormones, growth factors, cytokines, etc.), pathological (infections, inflammation, hepatectomy, etc.), genetic (polymorphism of expression or function) and environmental (drugs, dietary compounds, environmental pollutants) factors [2]. Changes in enzyme level may be initiated at several steps along the pathway from DNA to functional protein: transcription, pre-mRNA and mRNA processing (splicing, nuclear export, degradation), translation (protein synthesis), and post-translational processing (proteolytic degradation, phosphorylation, acetylation, etc.) [3], as illustrated by Fig. 1. Theoretically, regulation at any one of these steps could lead to differential production.

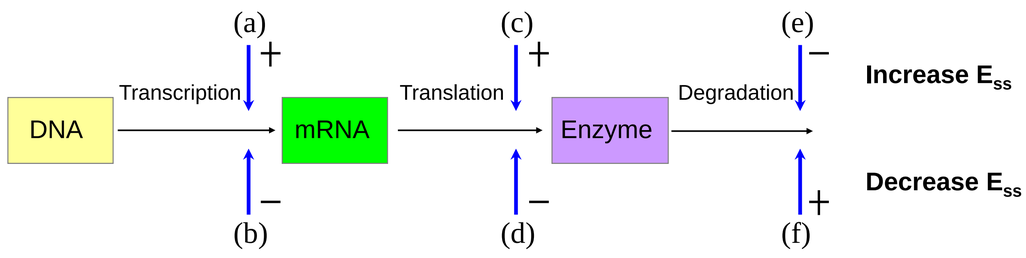

Fig. 1. A schematic of *in vivo* enzyme turnover. Increase of transcription (a), or increase of translation (c), or decrease of degradation can lead to the increase of enzyme level. Decrease of transcription (b), or decrease of translation (d), or increase of degradation can lead to the decrease of enzyme level.

Methods for Determining the Turnover of Hepatic CYPs

Various *in vitro* and indirect *in vivo* methods have been used to estimate the turnover of human hepatic CYPs, as summarized below:

In vitro methods

1) Radio-labeling of enzyme ('Pulse-chase' method)
2) Degradation of enzyme in cultured hepatocytes or liver slices
3) Induction of CYP enzymes in hepatocytes

In vivo methods

1) Recovery of enzyme activity after enzyme induction
2) Recovery of enzyme activity after mechanism-based inhibition (MBI)
3) Pharmacokinetic modeling of auto-induction

A summary of published hepatic CYP turnover values

Table 1 summarizes published estimates of the half-lives of various human hepatic CYPs based on the different *in vitro* and *in vivo* methods summarized above. Although there is some agreement between values derived using different approaches, there are also some marked discrepancies. For example, the estimate of average CYP3A4 half-life ranges from 26 to 140h.

Table 1. Turnover half-lives of human hepatic CYPs (updated from Ghanbari et al. 2006 [4]. NC = not clear). Details of the references can be found in the review submitted to *Current Drug Metabolism* (in press).

Enzyme	Method	n	$t_{1/2}$ (h) *
CYP1A2	*In vitro* Method 1	1	51
	In vitro Method 2	NC	43**
	In vitro Method 2	5	36 (8-58)
	In vivo Method 1	12	39 (27-54)
	In vivo Method 3	7	105
CYP2A6	*In vitro* Method 2	2	26 (19-37)
CYP2B6	*In vitro* Method 2	1	32
CYP2C8	*In vitro* Method 2	5	23 (8-41)
CYP2C9	*In vitro* Method 2	5	104
CYP2C19	*In vitro* Method 2	3	26 (7-50)
CYP2D6	*In vitro* Method 2	4	70
	In vivo Method 2	13	51
CYP2E1	*In vitro* Method 2	5	27 (7-40)
	In vivo Method 1	6	60
	In vivo Method 2	11	50 ± 19
CYP3A4	*In vitro* Method 1	1	44
	In vitro Method 2	NC	26**
	In vitro Method 2	4	79
	In vivo Method 1	15	72**
	In vivo Method 3	6	96 ± 38 (53-154)
	In vivo Method 1	7	72 (20-146)
	In vivo Method 1	3	(85-806)
	In vivo Method 1	8	(36-50)
	In vivo Method 3	13	10** (2-158)
	In vivo Method 3	35	94 (62-205)
	In vivo Method 3	7	70
	In vivo Method 3	16	85 ± 61
	In vivo Method 3	6	140 (48-284)
CYP3A5	*In vitro* Method 2	3	36 (15-70)

Determining the Turnover of Intestinal CYPs

Several CYP enzymes expressed in human liver have also been detected in the human small intestine, including CYP1A2, CYP2D6, CYP2E1, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5. These enzymes are located in mature enterocytes, which are localized mainly in the tips of the villi. Epithelial stem cells in the intestinal crypts divide and differentiate into enterocytes that migrate from the crypt base to the villus tip, where they are sloughed off into the gut lumen. The processes of enterocyte migration and maturation are closely linked.

The effective turnover of human intestinal CYP3A enzymes may be determined indirectly from studies using single doses of grapefruit juice, components of which selectively and irreversibly inhibit intestinal rather than hepatic CYP3A enzymes. Thus, following the oral administration of grapefruit juice the levels of active intestinal CYP3A enzymes may be followed with a selective probe such as midazolam. With this approach Greenblatt et al [5] estimated the average effective half-life of intestinal CYP3A at 23h. We have also analyzed data from three earlier studies with grapefruit juice [6-8] to give estimates of between 12 – 33h.

Conclusion

There are clear disparities in the estimates of the turnover of specific CYPs dependent on the method of assessment. If such estimates are to be incorporated into predictions of the *in vivo* impact of enzyme induction and MBI, further studies based on a consensus on methodology are needed. In the meantime, sensitivity analysis of predictions of induction and MBI to enzyme turnover should be an integral part of any modelling and simulation effort, and the use of selective values should be avoided.

References

[1] Correia, M.A. (2003) *Drug Metab. Rev.* **35**(2-3), 107-143.

[2] Maurel, P. (1996) *Adv. Drug Deliv. Rev.* **22**(1-2), 105-132.

[3] Pascussi, J.M. et al. (2004) *Xenobiotica* **34**(7), 633-664.

[4] Ghanbari, F. et al. (2006) *Curr. Drug Metab.* **7**(3), 315-334.

[5] Greenblatt, D.J. et al. (2003) *Clin. Pharmacol. Ther.* **74**(2), 121-129.

[6] Lundahl, J. et al. (1995) *Eur. J. Clin. Pharmacol.* **49**(1-2), 61-67.

[7] Takanaga, H. et al. (2000) *Clin. Pharmacol. Ther.* **67**(3), 201-214.

[8] Lilja, J.J. et al. (2000) *Clin. Pharmacol. Ther.* **68**(4), 384-390.

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