EPA Science Forum 2003
Partnering to Protect Human Health and the Environment

Emerging Technologies

The Importance Of Obtaining Information Prior To Determining V_{max}, K_m Values for Use In PBPK Models

Specific Content Of Tissue Enzymes Metabolizing Organophosphorus Pesticides

James B. Knaak[1], Rogelio Tornero-Velez[2], Fred Power[2], Jerry N. Blancato[2] and Curtis C. Dary[2],

[1]State University of New York at Buffalo, Buffalo, NY. [2]U.S. EPA, Human Exposure and Atmospheric Sciences Division, Las Vegas, NV.

ABSTRACT

PBPK/PD models require metabolism parameter values to represent certain tissue enzymes. Current literature values cannot be easily used in PBPK models because of differences in standard methodologies used in their determination. In practically all cases, the investigators failed to determine the specific enzyme content in tissues of interest. For example, the concentration of certain enzymes in plasma (PON1 paraoxonase) may vary as much as 10 fold (60 to 600 µg/ml) making it difficult to select proper enzyme-substrate concentrations for the determination of kinetic parameters (V_{max}, K_m). Recently, individual enzymes (CYPs 1A2, 2B6, 2D6 and 3A4) have been measured by spectral or immunochemical methods to obtain V_{max}, K_m values for human whole liver microsomes. Mathematical equations have been developed for expressing individual CYP specific content and activity in native liver microsomes for use in PBPK models.

BACKGROUND/OBJECTIVES

- Cytochrome p-450 isozymes (CYPs) located in liver microsomes catalyze the activation of Organophosphorous Insecticides (OP) to toxic, cholinesterase (ChE) inhibiting agents.
- A-esterases oxonases(PON1) located in brain, plasma and liver microsomes catalyze the hydrolysis of toxic agents to nontoxic hydrolysis products.
- The nature (CYP form) and specific content (concentration/unit of tissue) of these enzymes vary across species and within species making it difficult to obtain reproducible and representative metabolic rate constants.
- Advances in molecular biology provide a way to measure the specific content and activity of these enzymes in tissues.
- The objective of this poster is to present a sound approach to obtaining data required in PBPK/PD models (Figure 1).

METHODS

LIVER: P-450 ISOZYMES:

1. Determine the specific activity (V_{max}, K_m) of reconstituted human CYPs (1A2, 2B6, 2C9*1, 2C19 and 3A4)
2. Use Hanes-Woolf plot ([S]/v versus [S]) or Eadie-Hofstee plot (v versus v/[S]) to obtain V_{max}, K_m (Segel, 1993).
3. Express Vmax in pmol of metabolite per CYP/min and K_m in M.
4. Determine specific content of CYPs (1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) in human liver microsomes. Report content in pmol of CYP/mg of microsomal protein (see Table 1).
5. Normalize data for each CYP using the following equation (specific activity of CYP * specific content in microsomes) to give pmol of oxon, PNP, or TCP formed /min/mg of microsomal protein.

$$NR = \left[\frac{pmol}{\frac{\min}{pmolCYP}}\right] * \left[\frac{pmolCYP}{mg * protein}\right]$$

If multiple number of CYPs are involved calculate Total NR as indicated below:

TNR = ∑(pmol oxon/min/pmol CYP * pmol CYP/mg microsomal protein) + (...) + (...)

PLASMA or SERUM: A-ESTERASE:

6. Obtain plasma or serum from blood donors.
7. Determine the specific content of A-esterases in blood plasma using methods of Blatter-Garin et al., (1994), Costa et al., (1999) and Kujiraoka et al., (2000) involving the separation (ELISA) and immunoquantitation of PON1.
8. Determine the specific activity of A-esterases (PON1). Report activity in pmol/hr/kg of body weight.
9. Report activity in pmol/hr/kg of body weight for use in PBPK/PD models. Human liver contains an average of 32 mg of microsomal protein/g of liver (Lipscomb and Teuschler, 2000), while rat liver contains 47.5 mg of microsomal protein/g of liver (Gentest Corp).

DISCUSSION AND CONCLUSIONS

1. The specific content of individual CYPs, A-esterases, and PON1 in tissues varies considerably between individuals and is believed to be the main reason for the poor reproducibility of V_{max}, K_m values in and between laboratories. See Table 1.
2. The specific activity of hydrolytic enzymes in plasma (PON1, A-esterase) is well known. However, recent studies have indicated the specific content of these enzymes in plasma varies between certain individuals and needs to be determined.
3. The specific activity and content of A-esterases in liver microsomes need to be determined according to the methods used for the plasma enzymes.

REFERENCES

Blatter Garin M-C, Abbott C, Messmer S, Mackness M, Durrington P, Pometta D, James RW (1994) Quantification of human serum paraoxonases by enzyme-linked immunoassay: population differences in protein concentrations. Biochem J 304:549-554.

Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE (1999) The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. Chem-Biol Interaction 38:48.

Kujiraoka, T, Oka T, Ishihara M, Egashira T, Fujioka T, Saito E, Saito S, Miller NE, Hattori H (2000) A sandwich enzyme-linked immunosorbent assay for human serum paraoxonase concentration. J Lipid Res 41:1358-1363.

Lipscomb JC, Teuschler LK (2000) Integration of in vitro data on xenobiotic metabolism and tissue enzyme recovery with physiologically based pharmacokinetic (PBPK) modeling to estimate human interindividual pharmacokinetic variance. Annual Meeting of the Society of Risk Analysis. Washington, DC, December 2000.

Segel IH (1993) Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems. John Wiley & Sons, Inc., NY.

FIGURE & TABLE

FIGURE 1. PBPK MODEL FOR PARATHION

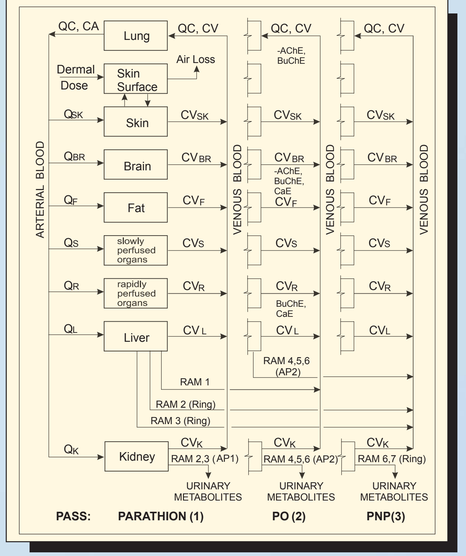

TABLE 1. Specific Content of CYPs in Native Human Liver Microsomes (HLM)

CYP	HLM-3	HLM-23	HLM-24	HLM-34	HLM-43	HLM-56	AVE
1A2*	7	50	85	77	23	35	46.2
2A6	83	20	56	54	70	30	52.2
2B6*	18	2	7	10	53	4	15.6
2C9	42	74	56	42	80	51	50.5
2C19	9	15	17	8	6	47	17.0
2D6	13	12	16	29	10	4	14.0
2E1	83	52	41	84	28	22	51.6
3A4*	95	38	85	79	306	94	116
3A5	0.9	0.5	0.72	1.00	1.1	0.7	0.82

Data taken from Gentest Corp., Woburn, MA.
Specific Content in pmol of enzyme per mg of protein

EPA SCIENCE FORUM 2003 MAY THIS MAY 7, 2003

Partnering to Protect Human Health and the Environment