



A COMPARISON OF CYTOCHROME P450 ACTIVITIES IN BACTOSOMES, SUPERSOMES, AND HUMAN LIVER MICROSOMES

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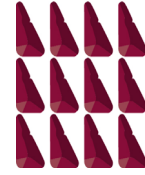
Abstract:

Recombinant cytochrome P450 enzymes (*r*CYPs) and human liver microsomes (HLMs) are used in *in-vitro* drug metabolism studies: notably, reaction phenotyping (enzyme mapping) and evaluation of the inhibitory effects of new chemical entities (NCEs). In the present study, we compared two panels of commercially available human *r*CYPs, one expressed in insect cells (Supersomes from Gentest) and the second in *E. coli* (Bactosomes from Cypex Ltd). We also compared their catalytic activities with those in a pool of human liver microsomes from Xenotech, LLC. Kinetic constants (K_m and V_{max}) were determined for each marker substrate reaction. V_{max} and enzymatic rate results are summarized here for each *r*CYP.



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Bactosomes from Cypex Ltd, Supersomes from Gentest Corporation, and pooled Human Liver Microsomes (product number H0610) from Xenotech, LLC were used in this comparison study under assay conditions defined in Xenotech's Standard Operating Procedures (SOPs). All rCYPs and HLMs were incubated in 50 mM potassium phosphate buffer (pH 7.4) containing 1 mM EDTA, 3 mM MgCl₂, 1 mM NADP(H), 5 mM glucose-6-phosphate, and 1 Unit/ml G-6-P-dehydrogenase.

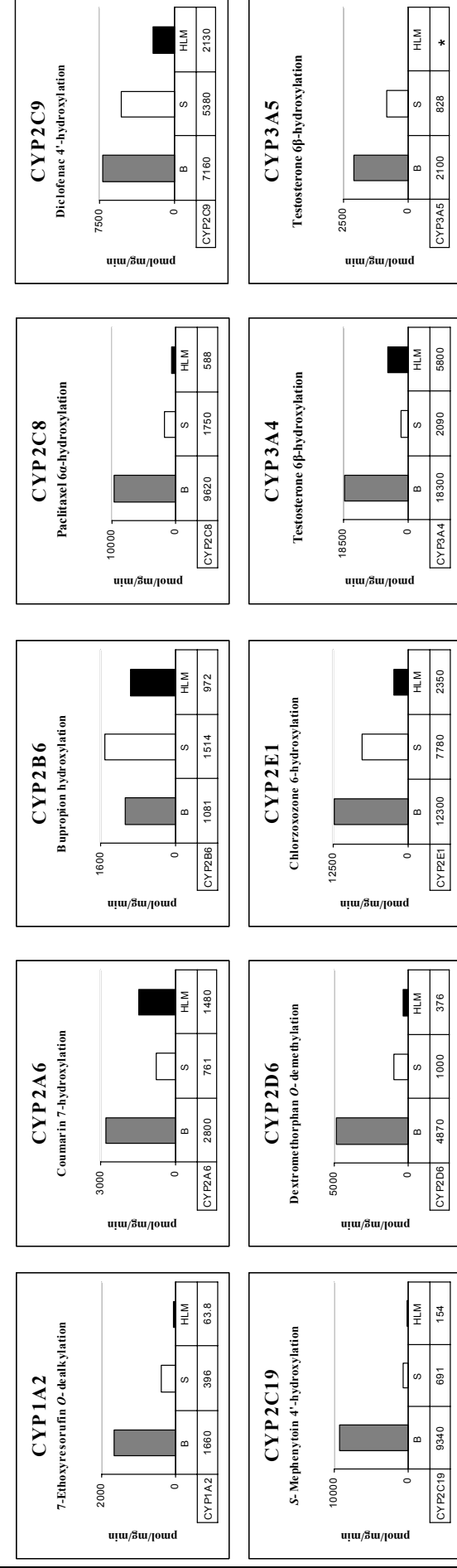
Introduction:

This study was designed to compare the activities of two types of commercially available human rCYPs; one expressed in insect cells, and the other in *E. coli*. Catalytic activities of both were compared with pooled human liver microsomes as a reference.

All rCYP preparations contained high levels of NADPH-CYP oxidoreductase (>1000 nmol cytochrome *c* reduced/mg/min) except for rCYP2C9 and rCYP3A5 (in the case of Bactosomes), and rCYP2A6, 2C9, 2C19, 3A4 and 3A5 (in the case of Supersomes). Cytochrome b₅ was co-expressed with rCYP2E1 in insect cells (Supersomes), but not *E. coli* (Bactosomes).

Figure 1: Enzymatic rates

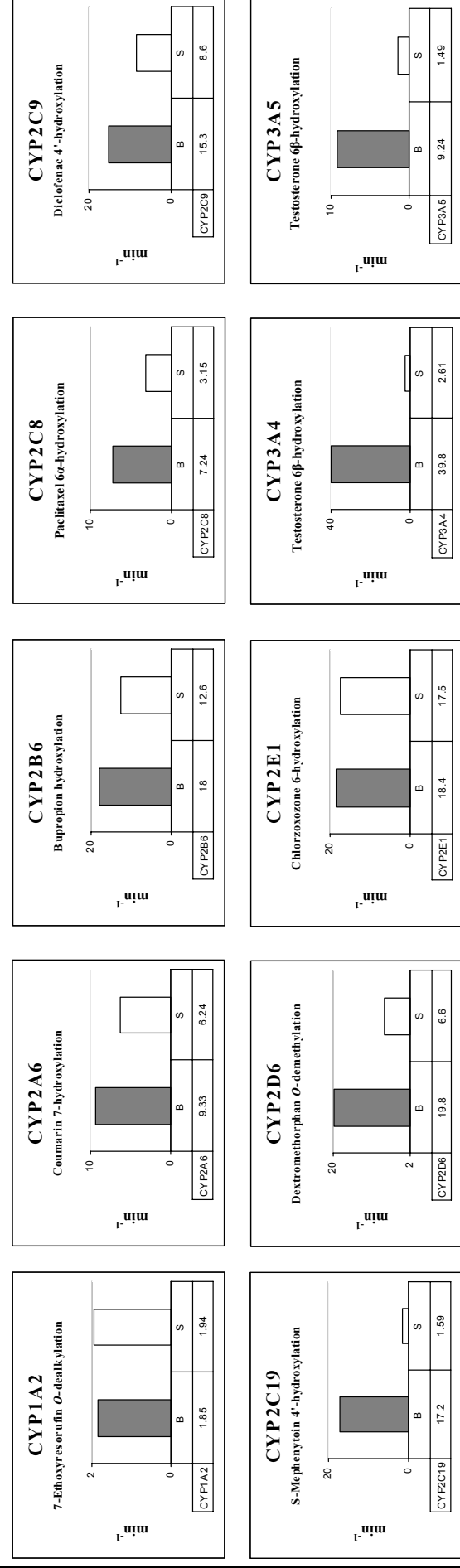
■ Bactosomes (B) □ Supersomes (S) ■ Pooled human liver microsomes (HLM)



* In human liver microsomes, testosterone 6 β -hydroxylation is catalyzed by both CYP3A4 and CYP3A5

Figure 2: Turnover number

■ Bactosomes (B) □ Supersomes (S)



Turnover number is mol metabolite formed per mol rCYP per min.

Results:

Figure 1 shows that V_{max} values (expressed on a per mg of protein basis) are greater in Bactosomes than Supersomes for all CYPs except CYP2B6.

Figure 2 shows that turnover numbers are greater for Bactosomes than Supersomes for all rCYPs except CYP1A2.

Figure 3 shows that the turnover number of rCYPs can be as much as 15 times greater in Bactosomes than in Supersomes.

Conclusions:

Under the conditions examined, nine of the ten rCYPs expressed in *E. coli* (Bactosomes) showed greater enzymatic activity compared with the same enzymes expressed in insect cells (Supersomes). Similarly, nine of the ten Bactosomes showed greater turnover numbers compared with Supersomes. These results demonstrate that human rCYPs expressed in *E. Coli* are as active, and frequently more active, than the corresponding rCYPs expressed in insect cells.

References:

- McGinnity DF, Griffin SJ, Moody GC, Voice M, Hanlon S, Friedberg T, Riley RJ: Characterization of the major drug-metabolizing human hepatic cytochrome P450 enzymes expressed in *Escherichia coli*. *Drug Metab Dispos*, 27(9):1017-1023, 1999.
- McGinnity DF, Parker AJ, Soars M, Riley RJ: Automated definition of the enzymology of drug oxidation by the major human drug metabolizing cytochrome P450s. *Drug Metab Dispos*, 28(11): 1327-1334, 2000.
- Crespi CL and Penman BW: Use of cDNA-expressed human cytochrome P450 enzymes to study potential drug-drug interactions. *Adv Pharmacol*, 43:171-188, 1997.

Bactosomes is a trademark of Cypex Ltd, Dundee UK
Supersomes is a trademark of Gentest Corporation, Woburn, MA

Figure 3: Bactosomes turnover, expressed as fold difference of Supersomes turnover

