

Superfund Basic Research Program Annual Meeting
Dartmouth College
November 9-12, 2003

ROLE OF THE CALPAIN PATHWAY IN ARSENITE-MEDIATED DECREASES IN CYP3A
IN CULTURED RAT HEPATOCYTES

Trisha L. Noreault¹, Barney E. Dwyer^{2,3}, Ralph C. Nichols^{2,4}, Heidi W. Trask⁵, Steven A. Wrighton⁶, Judith M. Jacobs², Peter R. Sinclair^{1,2,5} and Jacqueline F. Sinclair^{1,2,5}
Depts of Pharmacology/Toxicology¹, Neurology³, Microbiology/Immunology⁴, Biochemistry⁵,
Dartmouth Medical School, Hanover, NH 03755, ²VA Medical Center, WRJ, VT 05009; ⁶Lilly
Research Laboratories, Indianapolis, IN 46285

We have previously shown in cultured rat hepatocytes that arsenite, at non-toxic concentrations, decreases CYP3A induction by both dexamethasone and phenobarbital by a post-transcriptional mechanism. As found for CYPs 2B and 1A, we show here that addition of heme does not prevent the arsenite-mediated decreases in CYP3A in primary cultures of rat hepatocytes. We also investigated whether arsenite decreases CYP3A formation by increasing degradation of the CYP protein by the calpain pathway. Conditions were established for monitoring calpain activity in intact cells in serum-free medium, using t-butoxycarbonyl-Leu-Met-7-amino-4-chloromethylcoumarin as a substrate. Combined treatment with dexamethasone, arsenite and calpeptin, an inhibitor of the calpain pathway, resulted in greater decreases in CYP3A protein compared to treatment without calpeptin. None of the treatments altered protein synthesis or reduction of MTT, indicating no cytotoxicity. These results indicate that arsenite does not increase degradation of CYP3A protein via the calpain pathway. Further, our results suggest that a labile protein, whose degradation is inhibited by calpeptin, potentiates the effect of arsenite to decrease CYP3A. This work was supported in part by NIH-ES10462 and the Department of Veterans Affairs.