CHEMICAL MODIFICATION OF GLUTATHIONE S-TRANSFERASE PI: FATE OF PROTEIN EXPOSED TO ACETAMINOPHEN *IN VIVO*

Rosalind Jenkins, Neil Kitteringham, Christopher Goldring, Emma Dalton-Brown, Charlotte Hirst, Dominic Williams & Kevin Park. Dept Pharmacology, University of Liverpool.

Chemically reactive metabolites (CRMs) are thought to be pivotal in the development of many adverse drug reactions, but the level of covalent binding to proteins does not necessarily equate with the severity of a toxic response. This is exemplified by the fact that binding of hepatotoxic doses of paracetamol (APAP) to liver proteins is similar to its non-toxic congener 3'-hydroxyacetanilide (Rashed *et al.*, 1990). A fundamental understanding of how inherently reactive drug metabolites modify the structure and function of specific proteins in target organs is essential if we are to confirm the 'critical protein hypothesis of drug toxicity,' and to develop accurate *in vitro* screens for CRMs. We have characterised the chemical and functional changes in the putative target protein glutathione-S-transferase pi (GSTP) on *in vitro* exposure to the CRM of APAP, N-acetyl-p-benzoquinoneimine (NAPQI), and related covalent modification of Cys47 with inhibition of GSH conjugation to model electrophiles (Jenkins *et al.*, unpublished observations). The observations were repeated in a cell line engineered to overexpress GSTP and exposed to extracellular NAPQI (Jenkins *et al*., unpublished observations; Goldring *et al.*, 2006). Here we report on the status of GSTP in freshly-isolated, metabolically competent mouse hepatocytes (derived from male CD1 mice (25-30g)) exposed to either 0.35mM NAPQI or 5mM APAP. The GST activity was reduced by 65% (SD 7.94) in hepatocytes treated for 5h with APAP compared to control levels ($P<0.0005$, Student's T-test) and was linked to a reduction in the level of the protein rather than directly to the presence of an adduct on Cys47. Western blotting and two-dimensional gel electrophoresis indicated that the loss of GSTP protein was a selective event. The studies were extended to examine the GSTP status in livers from male CD1 mice (n=4) (25-30g) treated with an hepatotoxic dose (530mg/kg) of APAP. At 1h post-treatment, GSH levels were depleted by 90% compared to vehicle controls and the GST activity was 131.4% (SD 20.5) of control levels ($P<0.0005$, Student's T-test), probably due to transcriptional activation of the stress response by factors such as Nrf2 (Chan *et al.*, 2001). By 2h post-treatment, two animals exhibited a 53 and 28% reduction in GST activity whereas in the remainder, the activity was mildly elevated as at 1h (mean APAP-treated 87.2% (SD 31.9), controls 102% (SD 17.1), $P<0.005$ Student's T-test). Protein profiling again indicated that the loss in GST activity was due to a reduction in the level of GSTP in treated mouse liver: GSTP levels in the mice exhibiting a reduction in GST activity were 16.8-25.6% of those in controls at 2h. Further studies will determine the fate of the GSTP protein, whether the loss of the protein is secondary to covalent modification and whether the elimination of the protein is a common feature of drug-targeted proteins *in vivo*.

Chan, K *et al.* PNAS 2001; **98(8):** 4611-4616.
Goldring CE *et al*. Am J Physiol Cell Physiol 2006; **290(1):** C104-115.
Rashed MS *et al*., Drug Metab Dispos 1990; **18(5):** 765-770.