Chemical and biochemical aspects of thiophene bioactivation

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Thiophene rings are common constituents in drugs. The sulphur containing, aromatic, five membered heterocycle is beginning to be recognised as a potential toxiphore, due its ability to be oxidised to an epoxide or sulphoxide. These reactive metabolites are thought to be the cause of toxicities associated with several thiophene containing drugs. We aim to investigate the bioactivation of thiophene containing molecules and drugs and look at the effects of the reactive metabolites formed on the levels of certain biomarkers in liver and serum, such as taurine and ophthalmic acid, which have been suggested as biomarkers of liver injury and oxidative stress (Soga et al., 2006; Ishihara et al., 2006).

2-Phenylthiophene (2-PT) is a mono-substituted thiophene shown to be metabolised by induced (β-naphthoflavone) rat hepatic microsomes and human CYP1A1 supersomes to both an S-oxide and epoxide (Dansette et al., 2005), with S-oxide being the favoured route. We have shown that 2-PT undergoes extensive turnover in freshly isolated hepatocytes from non-induced rats. We identified two mono-oxygenated glutathione adducts (m/z 484 [M+H]+) and determined structure from their fragmentation pattern. The more polar, less abundant metabolite was identified as being formed from 2-PT S-oxide due to the presence of a fragment m/z 436 ([M+H-SO]). The less polar, more abundant metabolite was identified as being formed from 2-PT epoxide due to the presence of fragment m/z 466 ([M+H-H2O]). The ratio of metabolite formed from S-oxide to metabolite formed from epoxide was approximately 1:5.5. We also identified an S-oxide dimer (m/z 353). Concentration dependent cytotoxicity of 2-PT was observed in freshly isolated rat hepatocyte suspensions. The viability decreased from 95.44% ± 19.24% (6hr, 37°C, 10µM 2-PT) to 14.62% ± 7.8% compared to control (6hr, 37°C, 1mM 2-PT) as assessed by trypan blue exclusion.

The differing ratios of 2-PT epoxide and S-oxide formation in previous work (Dansette et al., 2005) in enzyme induced and the work presented here in non-induced animals suggest that induction of certain P450s can shift the metabolic profile of thiophenes, or that different P450 isoforms may be responsible for the two different metabolic pathways. To order to investigate this, we will utilise human liver epithelial cell lines engineered to overexpress different P450 isoforms, and to analyse the formation of 2-PT epoxide and 2-PT S-oxide and the resulting biochemical changes within the cell.

