Quantitative analysis of the Ah receptor signalling pathway.

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battery of genes, including the cytochrome P450 drug metabolising enzymes CYP1A1 and CYP1B1. These two P450s are known to demonstrate distinct cell type expression. Indeed, we have previously shown that CYP1B1 is a tumour-specific P450[1] and recently established several anti-cancer drugs as substrates for CYP1B1[2]. The aim of this study was to investigate the mechanism of cellular regulation of CYP1A1 and CYP1B1 by real time quantitative RT-PCR in three cell lines (MCF7, HEPG2 and MOG-G-CCM) known to differentially express CYP1A1 and CYP1B1 mRNA. In this investigation basal and inducible levels of CYP1A1 and CYP1B1 mRNA were determined for the three cell lines. The cells were exposed to the Ah receptor agonist 3-methylcholanthrene (3-MC) for a 12hr time period to determine the levels of inducible CYP1A1 and CYP1B1 mRNA. Specific primers and fluorescently labelled probes were designed for each cDNA, quantification of the mRNA was determined by the ABI7700 sequence detection system. Detection of the AhR and ARNT transcripts demonstrated basal levels for each amplicon, minimal changes in expression levels was observed on exposure to 3-MC. However, our results demonstrated differential basal and inducible expression of CYP1A1 and CYP1B1 in the cell lines. CYP1A1 basal expression was 3 and 5 fold higher in the HEPG2 cells compared to the MCF7 and MOG-G-CCM cells respectively. Where as basal CYP1B1 expression in the MCF7 and MOG-G- CCM cells was 500 and 4,000 fold higher than in the HEPG2 cells respectively. On exposure to 3-MC both CYP1A1 and CYP1B1 mRNA levels increased in all three cell lines. However the level of CYP1A1 mRNA in the MOG-G-CCM and CYP1B1 mRNA in the HEPG2 cell line exposed to 3-MC was below the threshold level for basal expression of the transcripts observed in the other two cell lines. Following exposure to 3-MC; MCF7 cells show inducible expression of both CYP1A1 and CYP1B1 protein, HEPG2 cells express CYP1A1 protein and MOG-G-CCM cells CYP1B1 protein. The results of this study suggest that a threshold of expression for CYP1A1 and CYP1B1 mRNA expression must be obtained before expression of protein occurs. This may indicate why although CYP1B1 mRNA is present in normal tissue the protein is not detectable.

References

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QUANTITATIVE ANALYSIS OF THE AH RECEPTOR SIGNALLING PATHWAY.

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The aryl hydrocarbon receptor (AhR) is a ligand activated receptor which on dimerisation with the aryl hydrocarbon nuclear translocator (ARNT) binds to xenobiotic responsive elements (XREs) that promotes the activation of a