Introduction

Many attempts have been made of late to quantify phase II metabolic activity in human skin, however due to the variety in approaches used, it is hard to gather many quantitative conclusions regarding the metabolic capacity of skin. With the aim of gaining a broader insight into the metabolic capacity of skin within the general population, a previously developed assay was used to enable quantitative analysis of the activity of four major phase two enzymes, NAT, UGT, COMT and SULT by the addition of substrate drugs and co-factors for each enzyme to freshly prepared skin incubated at 37°C. Following validation and optimisation studies, we were able to use the assay with confidence to analyse a large panel of patient skin samples (90). Skin was collected through Bradford’s Ethical Tissue bank over 2 years. Some skin samples were also supplemented with questionnaires designed to give a better insight into the patients skin history, for example previous or ongoing allergies, and use of topical drugs. The study is currently being expanded to include measurement of GST activity in patients using the substrate 2,4-dinitrochlorobenzene, DNCB.

Method

- A basic outline of the assay validated to quantify NAT, UGT, SULT and COMT is shown on the right.
- An 89 fraction was created by homogenising snap-frozen full thickness human skin from single donor.
- Immediately following centrifugation, 89 was extracted and incubated to 37°C. Substrate compound and co-factor were added and the mixture incubated for 4 hours. The reaction was stopped at time-points taken at 0, 1, 2, 4 hours.
- Metabolite concentrations at each time point were determined by analysing samples using UPLC-MS/MS, and plotted against time to produce a rate.
- Protein content was determined using the Bradford Assay and rate of production was calculated as ng/mg protein/hr.

Results

The activity levels of phase II metabolism are shown in the chart above, and have been sorted by rate of glucuronidation. A high degree of interpatient variability exists for each enzyme. The highest mean rate of activity was the sulphation of hydroxycoumarin (12.22ng/mg protein/hr), followed by glucuronidation (4.92ng/mg protein/hr), with the lowest being observed for the acetylation of procainamide with an average of 0.2ng/mg protein/hr. Methylation of dopamine was on average 2.27ng/mg protein/hr.

Ongoing work

In parallel to the measurement of GST levels in the patient samples, patient samples are also to be analysed for activity towards five further sensitising chemicals.

Conclusion and Future Work

The range and variation in the activity of phase II enzymes have been shown within a population. From this data, a better quantitative understanding of the range of clearance of compounds in the skin was obtained. We are currently looking to further expand our cohort to analyse patients who have shown contact sensitivity to various compounds with the hope of establishing whether a reduced ability to metabolise and clear xenobiotics is related to an increased risk of sensitivity.