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European Cells and Materials Vol. 32. Suppl. 4, 2016 (page 88) ISSN 1473-2262

Functional expression of xenobiotic metabolising enzymes in human skin and tissue engineered skin equivalents

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INTRODUCTION: The presence of xenobiotic metabolising enzymes in the skin is of considerable importance for the pharmaceutical/ cosmetic industries as these enzymes convert topically applied agents to either non-toxic or toxic metabolites that may lead to toxicity or hypersensitivity. The use of tissue engineered skin equivalents that replicate *in vivo* skin are extensively used in research and industry. However, whilst gene expression of xenobiotic metabolising enzymes in skin and skin equivalents has been detected by microarray analysis [1], data on protein expression and function of these enzymes in skin model systems is limited. Here we characterise gene expression and protein levels along with functionality of a sub-set of enzymes in whole skin and compare these to liver, commercially available and an in-house developed skin equivalent model to elucidate enzymatic profiles and activity.

METHODS: RNA and protein extracted from liver, whole skin, MatTek full thickness and our in-house skin models were used to determine gene expression by qPCR and protein levels using Western blotting. Enzyme activity and kinetics were assessed using commercially available assays.

RESULTS: From a selection of twelve enzymes examined qPCR revealed that liver expressed the most abundant levels of enzymes whilst, in general, whole skin expressed slightly more enzyme mRNA than skin models. However, ~90% of selected enzymes were increased in our in-house model compared to the commercial MatTek model. Similarly, Western blotting demonstrated protein expression for all except two of the twelve enzymes in whole skin. Decreased protein expression was observed in skin compared to liver, with the exception of FMO1, NAT1, COMT and GSTpi which showed increased expression (Fig. 1A). As an example, GST gene expression and protein levels from our in-house models is increased compared to MatTek models and equal to levels detected in skin (Fig. 1B). GST activity in our in house models (Fig. 1C) revealed similar levels of activity between the epidermal and dermal layers, indicating that this enzyme is uniformly distributed. GST specific activity peaked at 1 minute at a rate of 0.19 µmol/ml/min in the whole model.

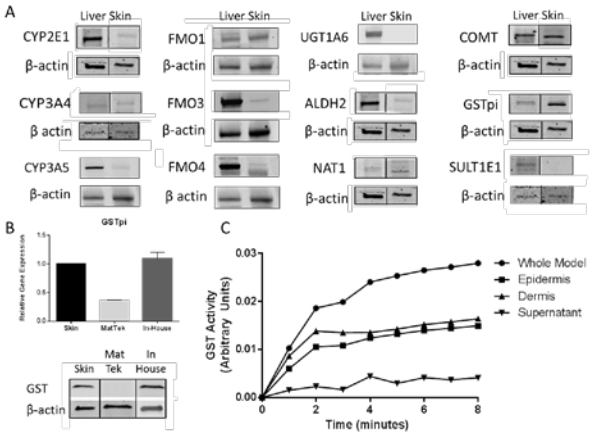

Fig. 1: Enzyme expression and activity levels. A. Protein expression in skin compared to liver. B. Gene expression, top, and protein levels, bottom, of GSTpi between skin and skin equivalents. C. GST activity in in-house skin models. GST specific activity in the whole model peaked at 1 min (0.19 µmol/ml/min).

DISCUSSION & CONCLUSIONS: Whilst at lower levels to liver, a high percentage of xenobiotic metabolising enzymes are expressed in the skin and skin equivalent models. We identified variations in enzyme expression between commercial and our in-house model. The main difference between these two models is the use of human dermis and not collagen as the scaffold in our in-house model. These data suggest that skin equivalents, in particular those based on dermis, will be a useful tool in investigating xenobiotic metabolism of topically delivered compounds.

REFERENCES: [1]F Oesch et al. (2014) *Arch Toxicol* **88**:2135-63.

ACKNOWLEDGEMENTS: This work was funded through a NC3Rs Phase 1 CRACK-IT award.