Quantification of Esterase Activity in Human Keratinocytes and Fibroblasts

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Cutaneous esterases play an important role in the activation of prodrugs and in the biotransformation of xenobiotics. For example, they allow prodrugs to penetrate the skin barrier as lipophilic compounds before being hydrolysed to the active compound to produce the therapeutic effect.

The aim of the study was to develop a quantitative assay measuring esterase activity in human keratinocytes and fibroblasts. Fluorescein diacetate (FDA), a non-fluorescent substrate, was chosen as a suitable metabolic probe, which is rapidly hydrolysed by esterases to the fluorescent product fluorescein. Primary cells were obtained from juvenile foreskin and grown on 96-well plates until they reached confluency. Following addition of increasing concentrations of FDA, fluorescein emitted fluorescence was monitored in a microplate reader over time. The specification of reaction time was of crucial importance for the determination of the enzyme kinetics. Initial reaction rates were used for the calculation of maximum transformation velocity (V_{max}) and Michaelis-Menten constant (K_m). As expected, a higher esterase activity was observed in keratinocytes which are superior in the cleavage of e.g. glucocorticoid esters. In conclusion, the assay for FDA hydrolysis provides an accurate, reliable, and reproducible *in vitro* test to determine esterase activity in human keratinocytes and fibroblasts. Future investigations will address the esterase activity in human skin *ex vivo* and commercially available 3D skin models.