HUMAN IN VITRO MODELS OF DERMAL METABOLISM
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Abstract

Dermal metabolism is recognized as an important consideration in evaluating the topical exposure of chemicals present in typical applied pharmaceutical and cosmetic products. Human skin placed in flow-through diffusion cells, skin homogenates, and isolated keratinocytes are some of the models that have been used to study the dermal metabolism of specific chemicals. The objective of this research was to establish human in vitro models for studying the dermal metabolism of chemicals. The metabolic capacity of freshly excised full-thickness human skin and human keratinocytes was evaluated using probe substrates for known xenobiotic metabolizing cytochrome P450 (CYP) enzymes (N-acetyl, glucuronidation, and sulfonylation) and esterases. Measurable CYP enzyme activities were observed for all transfectants and enzymes as well as for CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP2E1. Both freshly excised full-thickness human skin and keratinocyte exhibited a similar pattern of measurable enzyme activities, which correlated with enzyme activities previously reported in the literature. The effects of storage conditions, storage medium, and length of storage (up to 48 hours) on the metabolic capacity of freshly excised skin were also evaluated. The observed metabolic activities were well maintained by storage of the skin on wet ice for up to 48 hours. In conclusion, freshly excised human full-thickness skin and human keratinocytes are suitable models for evaluating potential dermal metabolism of chemicals.

Materials and Methods

Preparation and Storage of Human Skin. Frozen excised human abdominal skin from three donors was obtained from plastic bags on wet ice or in Belzer UW solution on wet ice immediately following excision, and the skin sample was transported to our facility. Portions of the skin were used in experiments either immediately or stored for 24 hours or 48 hours prior to use in experiments.

Processing of Skin. At each time point (Day 1, Day 2, and Day 3), skin was placed on a tray of ice covered with a plastic sheet with the epidermal side down. The fat layer and subcutaneous tissue beneath the dermis were removed with scalpel and scissors. Full-thickness skin punches were prepared using a 10-mm biopsy punch. The skin punches were stored in Belzer or supplemented Kico-Heinsaker buffer until the start of incubations.

Skin Punch Incubations. Various human CYP-specific substrates and conjugating enzyme-specific substrates were prepared in water or acetate buffer and diluted with supplemented Kico-Heinsaker buffer to dosing concentrations. Aliquots (1 µL) of the dosing solutions were transferred to uncoated 24-well plates. One skin punch was transferred to each well. The incubations were conducted at 37°C in 5% CO₂ on an orbital shaker in a humified incubator for 24 hours. The incubations were terminated with an equal volume of organic solvent and the samples were stored at −70°C until analysis. Data presented represent average data obtained with abdominal skin from three individual donors.

Keratinocyte Cultures and Incubations. Human Epidermal Keratinocytes (HEKa, Adult) were purchased from Cascade Biologics (Portland, OR). Keratinocytes were initially cultured in T-175 flasks and allowed to proliferate until 90-100% confluence based on supplier instructions. The cells were then plated in 48-well plates at 0.1 million cells/well and cultured until 80-100% confluence. The cultures were then incubated with 200 µL of various substrates in Krebs-Heinsaker buffer. The incubations were conducted at 37°C in 5% CO₂ on an orbital shaker in a humified incubator for 24 hours. The incubations were terminated with an equal volume of organic solvent and the samples were stored at −70°C until analysis.

Sample Analysis. The samples were analyzed by HPLC-UV or LC/MS methods to quantify the amount of metabolite formation.

Table 1: Overview of Enzyme Activities Evaluated in Full-Thickness Human Skin Punches and Keratinocyte Cultures

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Skin Punches</th>
<th>Keratinocyte Cultures</th>
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</thead>
<tbody>
<tr>
<td>CYP1A2 Phenacetin</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CYP2B6 S-mephenytoin</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CYP2C9 Tolbutamide</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CYP2C19 S-mephenytoin</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>N-Acetyl Transferase</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Sulfonyl Transferase</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Glucuronyl Transferase</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Esterase</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Figure 1: Metabolism in Full-Thickness Human Skin Punches (Day 1).

Figure 2: Metabolism in Full-Thickness Human Skin Punches (Day 2).

Figure 3: Metabolism in Full-Thickness Human Skin Punches (Day 3).

Results

The qualitative profiles of enzyme activities observed in full-thickness human skin punches and in cultured human keratinocytes were similar.

Keratinocyte cultures have been well characterized in vitro models for liver by evaluating full-thickness human skin and human epidermal keratinocytes in vivo.

- Metabolic activity in full-thickness human skin was maintained when stored on ice or in Belzer-UW solution for up to 48 hours.

- The qualitative profiles of enzyme activities observed in full-thickness human skin and human epidermal keratinocytes were similar.

- Metabolic activity in 48-hour full-thickness human skin punches was generally higher than the activity in 48-hour keratinocytes cultures.

- Full-thickness human skin punches appear to be a suitable model for the evaluation of the metabolism of xenobiotics.

References