HUMAN *IN VITRO* MODELS OF DERMAL METABOLISM

Aruna Koganti*, LaShawn Brown, Jonathan Smith, Danielle Mesa, Jennifer Bonass, Mark Fleischer, and Paul M. Silber*

BioreclamationIVT, 1450 South Rolling Road, Baltimore, MD

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Abstract

Dermal metabolism is recognized as an important consideration in evaluating the topical exposure of chemicals present in topically applied pharmaceutical and cosmetic products. Intact 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, transferases (N-acetyl, glucuronsyl, and sulfonyl) and esterases. Measurable CYP enzyme activities were observed for all transferases and esterases as well as for CYP1A2, CYP2B6, CYP2E1, and CYP3A4. Both freshly excised full-thickness human skin and keratinocytes exhibited a similar pattern of measurable enzyme activities, which correlated with enzyme activities previously reported in the literature (1). The effects of storage conditions, storage medium, (ice vs Belzer UW solution), 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 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.

Introduction

The skin is the largest organ in the body and the major protective barrier against environmental hazards. It possesses multiple functional activities such as xenobiotic metabolism, influx or efflux of xenobiotics into or out of its cells via transporter proteins, immunocompetency, and protection against UV radiation (2).

Xenobiotic metabolism by skin is an important consideration in the transdermal delivery of pharmaceuticals and cosmetics, as well as in the toxicity potential of environmental chemicals. Historically, the metabolic capacity of skin has been approached from the perspective of the chemical class of interest. For example, the metabolism of polycyclic aromatic hydrocarbons and the metabolism of steroids were studied widely using skin homogenates or by radiometric *in vivo* studies. More recently, intact viable skin in diffusion cells was used as a model for dermal metabolism (3). Recent studies have also evaluated the expression of CYP enzymes in skin cell cultures using RT-PCR (1, 4).

In the case of liver, which is the major metabolizing organ in humans, *in vitro* models such as liver slices, hepatocytes, liver microsomes, and liver S9 have been well established and used widely in the evaluation of xenobiotic metabolism. These models have been well characterized in terms of the enzyme systems present, Phase I (CYP enzymes) and Phase II (transferase enzymes). Thus, the objective of the current efforts was to parallel the *in vitro* models for liver by evaluating full-thickness human skin disks, human keratinocytes, human skin microsomes, and human skin homogenates as *in vitro* models for dermal metabolism.

Materials and Methods

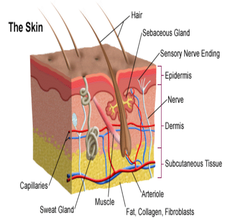
Procurement and Storage of Human Skin. **Freshly excised human abdominal skin from three donors was obtained from plastic surgeons with consent from the donors. The skin was stored either in a plastic bag on wet ice or in Belzer UW solution on wet ice immediately following excision, and the skin sample was transported to our facility. Portions of the skin were used in experiments either immediately or stored for 24 hours and 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 face down. The fat layer and subcutaneous tissue beneath the dermis were removed with scalpel and scissors. Full-thickness skin punches were prepared using an 8-mm biopsy punch. The skin punches were stored in Belzer UW solution or supplemented Krebs-Henseleit buffer until the start of incubations.**

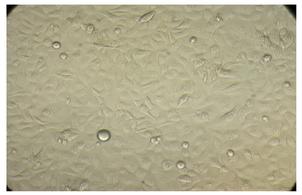
Skin Punch Incubations. **Various human CYP-specific substrates and conjugating enzyme-specific substrates were prepared in water or acetonitrile and diluted with supplemented Krebs-Henseleit buffer to dosing concentrations. Aliquots (1 mL) of the dosing solutions were transferred to uncoated 24-well plates. One skin punch was transferred to each well. The incubations were conducted at 37°C, in 5% CO_2, on an orbital shaker in a humidified 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.**

KeratinocyteCultures 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-Henseleit buffer. The incubations were conducted at 37°C, in 5% CO_2, on an orbital shaker in a humidified 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.**



Schematic Representation of Skin



Keratinocyte Culture

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

		Enzyme Activity in Skin Punches		Enzyme Activity in Keratinocyte Cultures	
Enzyme	Substrate	Present	Not Present	Present	Not Present
CYP1A2	Phenacetin	X		X	
CYP2A6	Coumarin		X		X
CYP2B6	S-mephenytoin	X		X	
CYP2C8	Paclitaxel		X		X
CYP2C9	Tolbutamide		X		X
CYP2C19	S-mephenytoin		X		X
CYP2D6	Dextromethorphan		X		X
CYP2E1	Chlorzoxazone	X		X	
CYP3A4	Testosterone	X			X
N-Acetyl Transferase	Para-Amino Phenol	X		X	
Esterase	Methyl Salicylate	X		X	
Glucuronyl Transferase	7-Hydroxy Coumarin	X		X	
Sulfonyl Transferase	7-Hydroxy Coumarin	X		X	

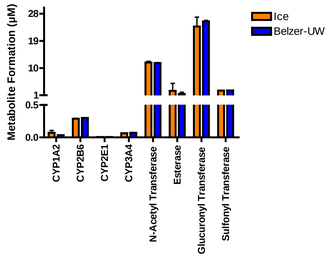


Figure 1: Metabolism in Full-Thickness Human Skin Punches (Day 1).

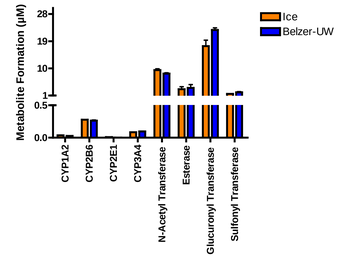


Figure 2: Metabolism in Full-Thickness Human Skin Punches (Day 2).

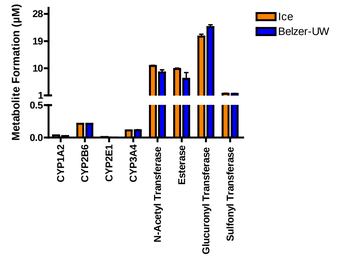


Figure 3: Metabolism in Full-Thickness Human Skin Punches (Day 3).

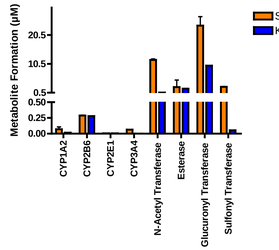


Figure 4: Metabolism in Full-Thickness Human Skin Punches vs Keratinocytes.

Results

•CYP1A2, CYP2B6, and CYP3A4 enzyme activities were measurable in full-thickness human skin punches. CYP2E1 enzyme activity also was detected, but was generally below the limits of quantification.

•The esterase and the phase II enzyme activities (glucuronyl transferase, sulfonyl transferase, and N-acetyl transferase) were observed at higher levels than CYP enzyme activity levels in full-thickness human skin punches.

•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 8-mm full-thickness human skin punches was generally higher than the activity in 48-well keratinocyte cultures.

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

References

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