Influence of Metabolism in Skin on Dosimetry after Topical Exposure

Robert L. Bronaugh, Steven W. Collier, Sara E. Macpherson, and Margaret E.K. Kraeling

Cosmetics Toxicological Branch, Food and Drug Administration, Washington, DC

Metabolism of chemicals occurs in skin and therefore should be taken into account when one determines topical exposure dose. Skin metabolism is difficult to measure *in vivo* because biological specimens may also contain metabolites from other tissues. Metabolism in skin during percutaneous absorption can be studied with viable skin in flow-through diffusion cells. Several compounds metabolized by microsomal enzymes in skin (benzo[*a*]pyrene and 7-ethoxycoumarin) penetrated human and hairless guinea pig skin predominantly unmetabolized. However, compounds containing a primary amino group (*p*-aminobenzoic acid, benzocaine, and azo color reduction products) were substrates for acetyltransferase activity in skin and were substantially metabolized during absorption. A physiologically based pharmacokinetic model has been developed with an input equation, allowing modeling after topical exposure. Plasma concentrations in the hairless guinea pig were accurately predicted for the model compound, benzoic acid, from *in vitro* absorption, metabolism, and other pharmacokinetic parameters. — Environ Health Perspect 102(Suppl 11):71–74 (1994)

Key words: skin absorption, metabolism, dosimetry, pharmacokinetic model

Introduction

The skin can serve as an important portal of entry of chemicals into the body. When systemic effects of a chemical are estimated after topical exposure, metabolism in skin must also be considered. Numerous studies have reported substantial activity of metabolic enzymes. These enzymes probably have an important role in the activation or elimination of chemicals absorbed into the body. Most enzymes found in other tissues are present in skin. Frequently the activity of these enzymes is less than the activity of the corresponding enzymes in the liver.

Only recently have investigators recognized the importance of skin's metabolic activity during percutaneous absorption. The lack of data is due to the difficulty in measuring skin metabolism *in vivo*. When body fluids are sampled, the contribution of skin cannot be separated accurately from metabolism in the liver and other organs. The advent of *in vitro* techniques for measuring absorption and metabolism has enabled investigators to more accurately assess the importance of skin metabolism.

The influence of skin metabolism is determined in part by the area of skin exposure. Skin is the largest organ of the body, with a surface area of 1.8 m^2 and a total weight estimated at 2.6 kg (*1*). Therefore, full body exposure could result in substantial metabolism during absorption, whereas application of the same dose of chemical to a small area of skin might saturate the enzymes, resulting in much less metabolism.

We have developed methods for measuring skin absorption and metabolism with an *in vitro* flow-through diffusion cell system. Using these procedures, we are investigating the effects of skin metabolism during percutaneous absorption. Results of some of these initial investigations indicate the extent to which skin metabolism can affect topical absorption. A physiologically based pharmacokinetic (PBPK) model has been developed to predict blood and tissue levels after percutaneous absorption of chemicals.

Historical Perspectives

Skin Metabolism of Polycyclic Aromatic Hydrocarbons

Many of the published studies on skin metabolism have dealt with the dermal toxicology of the polycyclic aromatic hydrocarbons (PAHs). These chemicals, which are found frequently in our environment, are potentially toxic to skin and other organs. The effects of these chemicals are relatively easy to study because they cause skin tumors that can be seen on test animals. Because metabolic activation of these compounds is required to produce toxic effects, researchers have focused attention on skin metabolism in toxicological studies.

Aryl hydrocarbon hydroxylase (AHH) is an important enzyme in the metabolism of PAHs. Its activity has been measured in the skin, liver, lung, and other tissues (*2*). The activity in skin exists primarily in the relatively thin, but metabolically active, epidermal layer (Table 1). AHH activity in liver is more than 10 times that of skin, regardless of whether the activity is expressed as milligrams of tissue protein, grams of tissue, or activity in the whole organ. AHH activity in lung is similar to that found in skin. Induction of enzyme activity by Aroclor occurs extensively in liver, skin, and lung.

The activities of AHH and other major enzymes involved in PAH metabolism in skin were compared with those found in rat liver (Table 2) (*3*). The specific activities of all five enzymes that were compared were higher in the liver, ranging from 4-fold higher activity for NADPH-cytochrome *c*-reductase to 185-fold higher activity for 7-ethoxycoumarin deethylase. Not only microsomal enzymes but also the soluble enzymes (epoxide hydratase and glutathione transferase) had lower activity in the skin.

We compared the activities of glutathione *S*-epoxide transferase in the liver, skin, and lung, using data from a series of publications by Mukhtar and Bresnick (*3–5*) (Table 3). Using mouse data, we found the liver is more than twice as active as the lung and more than 10 times as active as skin in metabolizing styrene oxide.

This paper was presented at the Workshop on Pharmacokinetics: Defining Dosimetry for Risk Assessment held 4–5 March 1992 in Washington, DC.

Address correspondence to R.L. Bronaugh, Cosmetics Toxicology Branch, Food and Drug Administration, Washington, DC 20204. Telephone (301) 344-5813. Fax (301) 344-0517.

Table 1. Aryl hydrocarbon hydroxylase activity in neonatal rat skin and its comparison with other tissue activities.

Tissue	Control			Arochlor
	pmoles/min/mg protein	pmoles/min/g tissue	pmoles/min/organ	pmoles/min/mg protein
Whole skin	0.7	18	12	11
Epidermis	0.6	13	4	12
Dermis	0.3	9	8	5
Liver	9.9	1262	676	39
Lung	0.8	19	4	7
Kidney	1.2	62	4	28
Carcass	0.3	11	40	1

Data represent the mean of three experiments and are expressed in terms of 3-hydroxide benzo[*a*]pyrene from benzo[*a*]pyrene. Modified from Mukhtar and Bickers (*2*).

Table 2. Effect of topical application of Aroclor on rat skin and liver enzymes.

Enzyme	Skin		Liver	
	Control	Aroclor	Control	Aroclor
Aryl hydrocarbon hydroxylase	1.1 ± 0.1	14.1 ± 1.3	43.1 ± 2.5	363 ± 9.8
7-Ethoxycoumarin deethylase	0.4 ± 0.1	8.1 ± 1.8	73.8 ± 8.4	224 ± 12.6
NADPH-cytochrome *c*-reductase	5.8 ± 0.6	6.9 ± 0.8	23.6 ± 1.2	29.4 ± 29.4
Epoxide hydratase	0.2 ± 0.03	0.2 ± 0.04	2.6 ± 0.3	5.0 ± 0.5
Glutathione *S*-transferase	3.2 ± 0.2	3.1 ± 0.3	23.4 ± 1.2	36.8 ± 2.6

Data are the mean ± SD for four animals. Specific activities (and substrates) were as follows: aryl hydrocarbon hydroxylase (benzo[*a*]pyrene) and ethoxycoumarin deethylase (ethoxycoumarin) activity was in pmole/min/mg of protein; cytochrome *c*-reductase (cytochrome *c*), epoxide hydratase (styrene oxide) and glutathione transferase (styrene oxide) were given as nmole/min/mg of protein. Modified from Mukhtar and Bickers (*2*).

Rat and mouse liver were equally active in this assay. Glutathione *S*-epoxide transferase activity in human skin was less than half that found in mouse skin.

Skin Metabolism of Steroids

The metabolism of steroids in skin has been extensively studied. In skin, the endogenous steroids testosterone and estradiol have pharmacological actions on hair growth and the rates of epidermal cell turnover. Anti-inflammatory steroids are probably the most widely used class of drugs for dermatological problems. Researchers are interested in how these drugs are activated, deactivated, and delivered across biological membranes (in the form of prodrugs) during metabolism.

In vitro studies with skin homogenates demonstrated that testosterone is converted to the 5α-hydroxy metabolite exclusively in skin, whereas in the liver this reaction results in the formation of both the α and β isomers (*6*). The importance of the skin enzymes in the metabolism of testosterone was further illustrated in the clinical studies of Mauvais-Jarvis et al. (*7*). Labeled testosterone was administered to four human volunteers by two different routes simultaneously: The ^{3}H isomer was applied topically and the ^{14}C isomer was administered intravenously (iv). The 5α and 5β metabolites of testosterone were determined in the urine and expressed as the 5α/5β ratio of androstanediols (primary metabolites) and 17-ketosteroids (secondary metabolites) (Table 4). The androstanediol data show that the tritiated metabolites from percutaneous absorption resulted in a 3-fold greater ratio of 5α/5β metabolites. These data confirm the earlier *in vitro* findings of a preference in skin for 5α metabolism of testosterone. Also, metabolism in skin during percutaneous absorption is sufficient to affect the pattern of systemic testosterone metabolites; hence, the different ratios of testosterone metabolites after iv and topical administration.

Table 3. Comparison of glutathione *S*-epoxide transferase activity in human and animal tissue.

Donor	Tissue		
	Skin	Liver	Lung
Human	3.2 ± 0.4	ND	ND
C_{57}Bl/6 mouse	7.7	83.8 ± 6.0	31.7 ± 1.3
Sprague-Dawley rat	ND	86.4 ± 2.6	ND

ND, not determined. Values are the mean ± SE (when available) of three to eight determinations. Specific activity was determined with styrene oxide as the substrate and is expressed as nmole/5 min/mg protein. Prepared from three papers by Mukhtar and Bresnick (*3–5*).

Skin Absorption and Metabolism Methods

Skin metabolism can be studied in combination with skin absorption measurements by using *in vitro* flow-through diffusion cells (*8*) with a physiologic buffer as the receptor fluid (*9*). Viability of the skin can be maintained in diffusion cells for at least 24 hr, as assessed by maintenance of glucose utilization and metabolic activity and by histologic evaluation. Hepes-buffered Hanks' balanced salt solution (HHBSS) is effective as a tissue culture medium (minimum essential medium) in maintaining glucose utilization, but phosphate-buffered saline solution results in the loss of glucose metabolism within 12 hr (Figure 1). The simplified formulation of a balanced salt solution may be advantageous during analysis of receptor fluid contents after an absorption and metabolism study. For this reason, we routinely used HHBSS as the receptor fluid in our studies.

Absorption and Metabolism Data

The influence of metabolism on percutaneous absorption has been examined in diffusion cells by using model compounds with different metabolic pathways.

Metabolism by P450 enzymes in skin during absorption was assessed in studies with four readily absorbed compounds: 7-ethoxycoumarin (*10*), benzo[*a*]pyrene (*10*), testosterone (*9*), and estradiol (*9*). The metabolism of 7-ethoxycoumarin to 7-hydroxycoumarin was small in humans and several species of rodents (Figure 2). Only 0.1% of the absorbed dose was metabolized in human skin, whereas 1.2% was metabolized with skin from the Sencar mouse.

Benzo[*a*]pyrene is metabolized to numerous compounds in skin and other organs. Metabolites formed in rodent skin were quantitated by thin-layer chromatography and identified by comparison with

Table 4. Metabolism of radioactive testosterone simultaneously administered intravenously (^{14}C) and percutaneously (^{3}H) in humans.

Male subject	5α/5β ratio of androstanediols		5α/5β ratio of 17-ketosteroids	
	Intravenous	Percutaneous	Intravenous	Percutaneous
JM	0.5	1.6	0.9	1.2
FC	0.4	1.2	0.9	1.7
AH	0.6	2.4	0.8	1.3
PM	0.5	1.3	1.0	1.1
Average	0.5	1.6	0.9	1.3

Values were determined from urine samples collected for a 3-day period. Modified from Mauvais-Jarvis et al. (*7*).

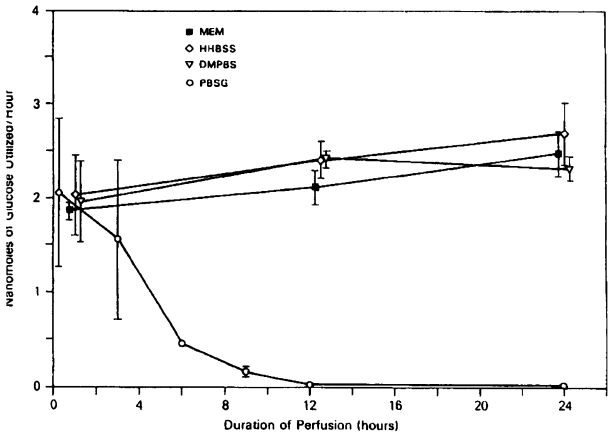

Figure 1. Rates of anaerobic glucose utilization of skin sections perfused over a 24-hr period. Skin sections from fuzzy rats were pefused in flow-through diffusion cells with Eagle's minimal essential medium, HEPES-buffered Hanks' balanced salt solution, Dulbecco's modified phosphate-buffered saline, or phosphate-buffered saline with 0.1% (w/v) glucose. Results are expressed as means ± SEM.

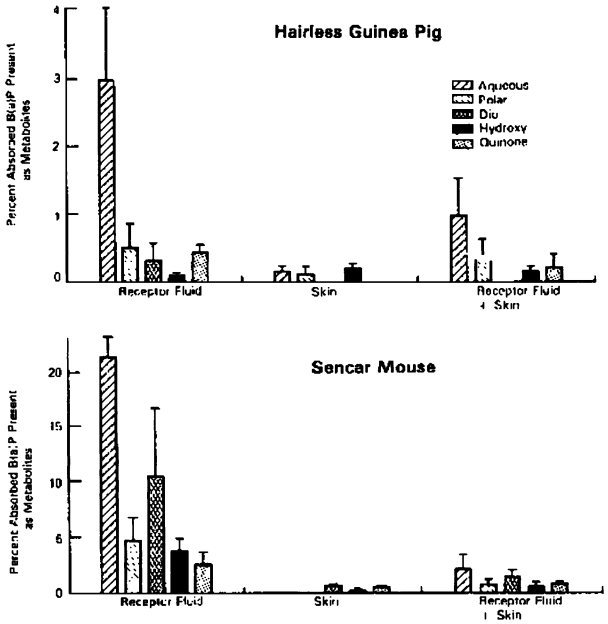

Figure 3. Percentage of absorbed benzo[*a*]pyrene metabolized by (*A*) hairless guinea pig and (*B*) Sencar mouse skin in 24 hr in flow-through diffusion cell. Values are means ± SEM of four or five determinations.

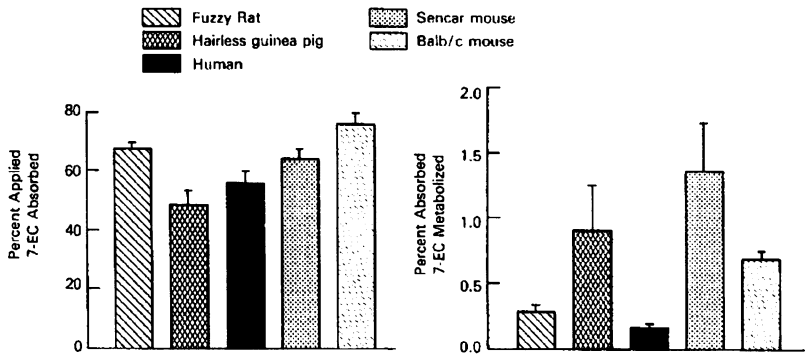

Figure 2. (*A*) Percentage of applied 7-ethoxycoumarin (7-EC) absorbed in 24 hr. (*B*) Percentage of absorbed 7-ethoxycoumarin metabolized in 24 hr by skin in flow-through diffusion cells. Values from two to four skin samples from each experimental subject were averaged and constitute one experiment. Values are means ± SEM of two or three experiments. Rat, guinea pig, and human skin were 200-μm sections; mouse skin was full thickness.

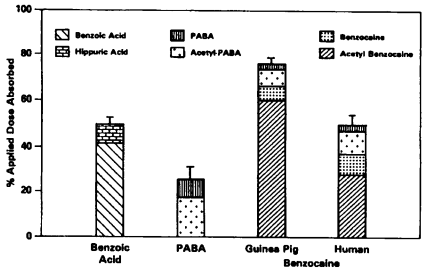

Figure 4. Percentage of applied compound absorbed and percentage of absorbed compound metabolized for benzoic acid and derivatives. Experiments were conducted for 48 hr with unabsorbed material washed from the surface of the skin at 24 hr. PABA, *p*-aminobenzoic acid.

standards. The aqueous fraction probably contains polar conjugates of benzo[*a*]-pyrene that cannot be extracted into ethyl acetate. When total metabolism is expressed in terms of the absorbed dose, we obtained about 4% (hairless guinea pig) and 8% (Sencar mouse) (Figure 3).

Testosterone and estradiol were more completely metabolized than the previous two compounds during skin permeation in the rat (*9*). Approximately 20% of the absorbed estradiol was converted to estrone. Testosterone metabolites totaled more than 30% of the absorbed material and cochromatographed with standards for 5α-androstane-3,17-diol; 4-androstane-3,17-dione; and 5α-dihydrotestosterone.

Additional biotransformation reactions were observed in hairless guinea pig skin with benzoic acid, *p*-aminobenzoic acid (PABA), and benzocaine (Figure 4) (*11*). A small amount of absorbed benzoic acid (approximately 7%) was converted to the glycine conjugate of benzoic acid (hippuric acid) during percutaneous absorption. Acetylation of primary amino groups can occur extensively in skin. PABA and benzocaine were substantially converted to the acetylated metabolite during absorption. Benzocaine was also a substrate for esterase, but because the conversion of benzocaine to PABA was small, it is a poor substrate for this enzyme. Metabolism of benzocaine was similar in human and hairless guinea pig skin.

The absorption and metabolism of benzocaine were examined in the hairless guinea pig after application in a water-soluble gel (*12*). A radiotracer dose (2 μg/cm^2) was compared with a 20-fold larger dose used to approximate concentrations of benzocaine applied for topical anesthesia. At the low dose, 80% of absorbed benzocaine was metabolized to *N*-acetylbenzocaine. At the high dose, saturation of skin acetyltransferase limited *N*-acetylbenzocaine formation, but 47% of the absorbed benzocaine was acetylated.

In preliminary studies, azo colors were observed to undergo azo reduction during

skin absorption (*13*). Several azo compounds that are simple derivatives of aniline were synthesized and found to be metabolized to aniline during azo reduction. The aniline was further biotransformed to other compounds with the *N*-acetylated derivative acetanilide as the most predominate.

PBPK Model—Dermal Input

A PBPK model is being developed for predicting *in vivo* blood and tissue concentrations of a chemical after topical exposure (*14*). *In vitro* skin absorption and metabolism of benzoic acid were measured with viable hairless guinea pig skin in a diffusion cell and described mathematically for input into the PBPK model. Tissue-plasma partition coefficients and Michaelis-Menton metabolic constants (K_m, V_{max}) were determined by *in vitro* techniques. Predicted blood levels after three different topical doses of benzoic acid agreed closely with *in vivo* measurements. A PBPK model may be useful for determining the influence of metabolism at the absorption site on the systemic effects of a toxicant.

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