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CARCINOGEN METABOLISM IN HUMAN SKIN GRAFTED ONTO ATHYMIC NUDE MICE: A MODEL SYSTEM FOR THE STUDY OF HUMAN SKIN CARCINOGENESIS

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Summary: Human skin grafted onto athymic nude mice maintains its major histological features and may provide a useful system with which to assess the carcinogen interaction with human skin. Significant differences were observed in basal levels of cytochrome P-450 and cytochrome P-448-dependent monooxygenase activities between human grafted and nude mouse epidermis. Topical application of crude coal tar (CCT) to human skin transplanted onto nude mice resulted in 3.9 & 3.5; 3.2 & 2.9 and 1.1 & 1.2 fold increases in mouse and human epidermal aryl hydrocarbon hydroxylase (AHH), ethoxyresorufin deethylase (ERD) and ethoxycoumarin deethylase (ECD) activities, respectively. CCT applied topically to mouse skin resulted in 27.8 & 6.4; 12.8 & 3.3 and 1.7 & 2.6 fold increases in mouse and human epidermal AHH, ERD and ECD activities, respectively. Topical application of coal tar either onto human transplanted skin or to mouse skin also resulted in substantial induction of hepatic and pulmonary AHH and ERD activities. These studies indicate that human skin grafted onto nude mice preserves its metabolic capacity and offers a useful model system with which to assess the effects of polycyclic aromatic hydrocarbons and CCT on cutaneous xenobiotic metabolism in the human population.

Clinical and epidemiological studies indicate that chemical carcinogens in the environment play a major role in the induction of human skin cancer (1,2). It is known that cutaneous tumors can be induced in experimental animals by topical application of chemical carcinogens such as PAHs including BP and DMBA (1,2). Although these animal studies have provided extensive information concerning mechanisms of carcinogenesis their relevance to human cancer has not been defined. Therefore model systems for the evaluation of carcinogen interaction with human tissues are needed as an approach to assessing risk of cancer in humans.

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Abbreviations used: BP, benzo(a)pyrene; 3-OH BP, 3-hydroxy benzo(a)pyrene; RF, resorufin; 7-HC, 7-hydroxycoumarin; PAH, polycyclic aromatic hydrocarbon; DMBA, 7,12-dimethylbenz(a)anthracene; AHH, aryl hydrocarbon hydroxylase; ERD, ethoxyresorufin O-deethylase; ECD, ethoxycoumarin O-deethylase; DTT, dithiothreitol; BSA, bovine serum albumin; DMSO, dimethylsulfoxide; CCT, crude coal tar.

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Rygaard (3) have shown that human skin grafts are accepted by athymic nude mice. Subsequent studies have shown that when human skin is transplanted onto athymic nude mice its main histological features are preserved even several months after transplantation (4). This model system has provided useful information concerning the percutaneous absorption of drugs and chemcials in humans (5,6). Because of the ethical considerations the use of known carcinogens for human studies is not feasible. However the human/mouse skin system does offer a useful alternative approach for such studies. Since this model system may provide useful information relating to enzymic constitution of human skin we have explored the cytochrome P-450 dependent-monooxygenases in human skin grafted onto nude mice. Cytochrome P-450-dependent monooxygenases metabolize a variety of exogenous and endogeneous compounds including drugs, carcinogens, and steroids (7) and our prior studies have verified their presence and inducibility in human skin (8).

MATERIALS AND METHODS

Chemicals: Gold label BP, ethoxycoumarin and resorufin were obtained from Aldrich Chemical Co (Milwaukee, WI). 7-Ethoxyresorufin was purchased from Pierce Chemicals, BSA, NADH and NADPH were obtained from Sigma Chemical Co. (St. Louis, MO). 7-Hydroxycoumarin was a product from ICN, K and K Laboratories, Plainview, NY. Standard CCT solution (USP) was used. All other chemicals were obtained in the purest form commercially available.

Grafts and mice: Male athymic nude mice (strain Balb/C, Harlan Industries, Indianapolis, IN) weighing approximately 30 g each were used. Mice were housed in a pathogen-free environment provided by laminar-flow air stations (Lab Products, Garfield, NJ) and maintained on an artificial circadian rhythm (12 hr darkness, 12 hr light) at room temperature (26°C). Human skin grafts for the nude mice were obtained from normally discarded surgical specimens from elective abdominoplasties. To permit successful growth of the graft, only the superficial skin was transplanted. The thickness of the transplanted skin was approximately 0.7 mm, and was obtained with a dermatome (Brown electrodermatome, model 901, Warsaw, IN). As described previously, the grafts were positioned on the left side laterally and toward the anterior end of the mouse (9). Experiments were initiated six weeks after engraftment.

Treatment of animals: Twenty four mice with skin engrafted from one human specimen were divided into three groups of eight animals. Animals of Group 1 were treated topically with 0.1 ml of CCT to the human grafted skin. Animals in Group 2 received 0.1 ml of CCT at a site of the animals opposite to the grafted site. Since the human grafts were only 2 cm in diameter, to deliver the dose of 0.1 ml CCT, it was applied in 10 split doses at intervals of 15 seconds. Animals in Group 3 received 0.1 ml of acetone on both engrafted human and mouse skin. All animals were killed 24 hrs after the topical application of CCT.

Preparation of enzyme source: The animals were killed by cervical dislocation. First the human skin grafts were carefully excised followed by the mouse skin, liver and lung. Tissues were washed with chilled 0.1 M phosphate buffer (pH 7.4). The skin was scraped with a sharp scalpel blade (Bard-Parker No. 10) to remove subcutaneous fat

and muscle and was minced with scissors in 0.1 M phosphate buffer (pH 7.4) containing 10 mM EDTA, 10 mM DTT and 20% glycerol. The minced tissues were homogenized with a Polytron tissue homogenizer (Brinkman Instruments, Switzerland) equipped with ST-10 generator. Post-mitochondrial fraction of skin was prepared by a centrifugation technique described earlier (10). Liver and lung post-mitochondrial fractions were prepared as described previously (11).

Enzyme assays: AHH activity was determined by a modification of the method of Nebert and Gelboin (12), the details of which were described earlier (10). The quantitation of phenolic BP metabolites was based on comparison with fluorescence of a 3-OH BP standard. ECD activity was determined according to a slight modification of the procedure of Greenlee and Poland (13), the adaptations of which were described earlier (10). ERD activity was determined by a modification of the method of Pohl and Fouts (14). A typical reaction mixture in a final volume of 1.25 ml contained 0.1 M phosphate buffer (pH 7.4), 0.96 mM NADPH, 2 mg BSA and 0.3 to 0.7 mg protein. The reaction was initiated by the addition of 1.5 μM ethoxyresorufin in 5 μl DMSO and was incubated for 10 min (liver) and 30 min (skin, lung) at 37°C, in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 2 ml methanol and the fluorescence of the deethylated product, resorufin was measured at excitation and emission wavelengths of 550 nm and 585 nm, respectively. Protein was estimated according to the method of Lowry et al (15) using BSA as a reference standard.

RESULTS AND DISCUSSION

Many chemical carcinogens including the PAHs require metabolic activation to elicit their carcinogenicity (16). AHH, a cytochrome P-450-dependent enzyme, is the first step in the metabolic activation of PAHs to reactive electrophilic intermediates which subsequently bind to DNA to initiate the process of carcinogenesis (16). The data in Table 1 provides a comparison of the basal levels of AHH, ERD and ECD activities in nude mouse skin and in engrafted human skin. The catalytic activities of AHH and ERD, related to a specific isozyme of cytochrome P-450, known as cytochrome P-448 (17), were found to be 1.3 and 1.4-fold higher in mouse skin as compared to human skin. Hydroxylation of ethoxycoumarin which is catalysed by both cytochrome P-450 and P-448 isozyme(s), was found to be 10-fold higher in mouse skin as compared to human skin. These differences in basal monooxygenase activities between human and mouse skin suggests that human skin contains predominantly the cytochrome P-448 isozyme rather than cytochrome P-450. This could be related to the continuous human exposure to low levels of environmental pollutants such as the PAHs including BP. Furthermore our data confirm that human skin is capable of metabolizing a variety of xenobiotics even several weeks after engraftment onto nude mice.

CCT, which is rich in PAHs, is a known skin carcinogen in experimental animals and in humans and yet is widely employed therapeutically in dermatologic practice,

Table 1: Effect of topical application of CCT to engrafted human skin on monooxygenase activities in engrafted human and mouse skin

Skin Site	AHH (pmol 3-OH-BP/ min/mg protein)	ERD (pmol RF/ min/mg protein)	ECD (pmol 7-HC/ min/mg protein
MOUSE SKIN			
Control	0.59 ± 0.03	0.18 ± 0.02	3.04 ± 0.14
CCT-Treated	9.82 ± 0.53 (16.6)*	1.17 ± 0.09 (6.5)*	3.59 ± 0.27 (1.2)
HUMAN SKIN			
Control	0.45 ± 0.03	0.13 ± 0.01	0.26 ± 0.02**
CCT-Treated	2.29 ± 0.12 (5.1)*	0.58 ± 0.03 (4.5)*	0.91 ± 0.08 (3.5)*

For treatment and other details see "Materials and Methods".
Data are expressed as mean ± SEM of three samples.
Values in parentheses indicates the fold increase over respective controls.
*Significantly higher than respective controls, $p < 0.01$.
**Significantly lower than control mouse skin, $p < 0.01$.

particularly in the treatment of chronic dermatoses such as eczematous dermatitis and psoriasis (18). The chronic use of medications containing CCT for extended periods of time has been associated with the development of skin cancer in human populations. Hodgson (19) reported that an individual treated twice daily for 7 years with application of a drug containing large amounts of CCT developed cutaneous squamous cell carcinoma. Stern et al (20) have shown that psoriatic individuals repeatedly treated with CCT are at increased risk for cutaneous cancer. There is evidence to suggest that tumor induction effect of CCT is associated with one or more of the several PAHs present therein (21). Our prior studies have shown that topical application of CCT to human skin and to mouse skin bearing UV-B induced squamous cell carcinomas results in substantial induction of cutaneous AHH activity (22,23).

In the data reported here topical application of CCT to human skin engrafted onto athymic nude mice resulted in induction of AHH and ERD activities in mouse skin (6.5-16.6 fold) and in human skin (4.5-5.1 fold) (Table 1). Topical application of CCT to mouse skin resulted in induction of AHH and ERD activities in mouse skin

Table 2: Effect of topical application of CCT to mouse skin on monooxygenase activities in engrafted human and mouse skin

Skin Site	AHH (pmol 3-OH-BP/ min/mg protein)	ERD (pmol RF/ min/mg protein)	ECD (pmol 7-HC/ min/mg protein
MOUSE SKIN			
Control	0.59 ± 0.03	0.18 ± 0.02	3.04 ± 0.23
CCT-Treated	16.45 ± 1.14 (27.8)*	2.30 ± 0.18 (12.8)*	5.29 ± 0.31 (1.7)*
HUMAN SKIN			
Control	0.45 ± 0.03	0.13 ± 0.01	0.26 ± 0.02**
CCT-Treated	2.90 ± 0.19 (6.4)*	0.43 ± 0.03 (3.3)*	0.68 ± 0.03 (2.6)*

For treatment and other details see "Materials and Methods".
Data are expressed as mean ± SEM of three samples.
Values in parentheses indicates the fold increase over respective controls.
*Significantly higher than respective controls, $p < 0.01$.
**Significantly lower than control mouse skin, $p < 0.01$.

(12.8-27.8 fold) and in the human skin grafts (3.3-6.4 fold) (Table 2). Topical application of CCT to the skin of neonatal rats or to SKH-1 hairless mice results in induction of epidermal ECD activity (23,24), however in the present study no effect of CCT on skin ECD activity in mouse skin was observed while the enzyme in human grafted skin was found to be slightly induced (2.6 and 3.5 fold) following topical application of CCT either to mice skin or to human skin (Table 1 and 2). These results suggest that human and mouse epidermal cytochrome P-450 isozymes are not similar and that the inducibility of catalytic activities of cytochrome P-448 isozymes is higher in mouse skin as compared to human skin.

The data in Table 3 and 4 indicate that there is substantial percutaneous absorption of CCT following topical application to either mouse or engrafted human skin. Thus CCT treatment of either mouse or human grafted skin resulted in the induction of mouse hepatic AHH (2.9-3.8 fold), ERD (6.8-12.1 fold) and ECD (2.5-3.8 fold) activities (Table 3) and induction of pulmonary AHH (3.5-3.9 fold) and ERD (2.9-3.2 fold) activities in the mouse (Table 4). These findings indicate that irrespective

Table 3: Effect of topical application of CCT to mouse skin or to engrafted human skin on monooxygenase activities in mouse liver

Groups	AHH	ERD	ECD
	(pmol 3-OH-BP/ min/mg protein)	(pmol RF/ min/mg protein)	(pmol 7-HC/ min/mg protein
Control	34 ± 3	11 ± 1	111 ± 8
CCT-Treated Mouse Skin	128 ± 10 (3.8)*	133 ± 11 (12.1)*	429 ± 22 (3.8)*
CCT-Treated Human Skin	97 ± 7 (2.9)*	75 ± 8 (6.8)*	281 ± 18 (2.5)*

For treatment and other details see "Materials and Methods".
Data are expressed as mean ± SEM of three samples.
Values in parentheses indicates the fold increase over controls.
*Significantly higher than respective controls, $p < 0.01$.

of whether CCT is applied to mouse or to human grafted skin, it penetrates sufficiently to elicit its effects on hepatic and pulmonary monooxygenases in the mouse skin. The studies of Reifenrath et al (6) have shown that percutaneous absorption in human skin grafted to nude mice is consistently higher than that determined in human skin in vivo. This may be due to lack of complete dermis in the graft. However, it has been suggested that human epidermis transplanted onto nude mouse retains its proliferative and barrier functions (6,9). In conclusion, our data

Table 4: Effect of topical application of CCT to mouse skin or to engrafted human skin on monooxygenase activities in mouse lung

Groups	AHH	ERD	ECD
	(pmol 3-OH-BP/ min/mg protein)	(pmol RF/ min/mg protein)	(pmol 7-HC/ min/mg protein
Control	1.54 ± 0.09	2.81 ± 0.16	15.6 ± 1.1
CCT-Treated Mouse Skin	6.03 ± 0.39 (3.9)*	9.04 ± 0.45 (3.2)*	17.7 ± 1.4 (1.1)
CCT-Treated Human Skin	5.32 ± 0.43 (3.5)*	8.18 ± 0.44 (2.9)*	18.8 ± 1.2 (1.2)

For treatment and other details see "Materials and Methods".
Data are expressed as mean ± SEM of three samples.
Values in parentheses indicates the fold increase over controls.
*Significantly higher than respective controls, $p < 0.01$.

indicate that the human-nude mouse skin model system offers a useful approach for the assessment of the pharmacologic properties of human skin.

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