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INDUCTION OF UDP-GLUCURONYLTRANSFERASE AND ARYLHYDROCARBON HYDROXYLASE ACTIVITY IN MOUSE SKIN AND IN NORMAL AND TRANSFORMED SKIN CELLS IN CULTURE

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Abstract—Methods have been developed which allow quantitative determination of UDP-glucuronyltransferase (UDPGT) and arylhydrocarbon hydroxylase (AHH) activities in unfractionated mouse skin. These methods were used for comparative studies of basal and induced enzyme activities in whole skin and cultured skin cells. After topical application of Aroclor 1254 to the skin UDPGT activities towards 1-naphthol, 3-hydroxybenzo[*a*]pyrene and benzo[*a*]pyrene-7,8-dihydrodiol were increased 3-fold and AHH activity was increased 15-fold. Topical application of the inducer also led to a marked increase of these enzyme activities in liver. UDPGT activity towards 1-naphthol was comparable in whole skin and in cultured keratinocytes and fibroblasts. In contrast, AHH activity was higher in cultured keratinocytes than in skin. In transformed epithelial cell lines the pattern of drug metabolizing enzymes was altered: UDPGT activity was increased 4- to 6-fold whereas AHH activity was decreased. However, AHH activity was still inducible by benz[*a*]anthracene or 7,12-dimethylbenz[*a*]anthracene in cultured cells. The altered pattern of AHH and UDPGT in transformed epithelial cell lines is consistent with toxin-resistance of initiated cells, similar to the toxin-resistance phenotype characterized in liver after initiation of hepatocarcinogenesis.

Mouse skin is widely used for testing the carcinogenic potential of chemicals and for investigating factors involved in initiation, promotion and progression of carcinogenesis. Since drug metabolizing enzymes are involved in the activation and inactivation of most carcinogens, a number of investigators studied the regulation of these enzymes in mouse skin and its relationship to carcinogenesis [1–7]. In addition, the metabolism of carcinogenic aromatic hydrocarbons was extensively studied in nontransformed and transformed skin cells [8–13].

Skin is a heterogenous organ and cumbersome to work with. This may be the reason why, despite numerous studies on drug metabolizing enzymes, quantitative and comparative studies on their regulation in skin cells *in vivo* and *in vitro* are scarce. However, this information is necessary to be able to relate results obtained with cultured cells to those obtained in whole skin. In the present study an attempt was made to compare in whole skin and primary cultures of keratinocytes and fibroblasts the regulation of AHH§ and UDPGT, two major phase I and II enzymes involved in the metabolism of carcinogenic aromatic compounds.

The pattern of drug metabolizing enzymes may be altered after initiation of carcinogenesis in skin, similar to observations in liver [14–19]. The altered pattern, i.e. decreased drug oxidation and increased drug conjugation, is consistent with increased toxin resistance of initiated hepatocytes. The toxin resistance phenotype leads to a growth advantage of initiated cells and may explain some types of tumor promotion in liver [20]. Because of its possible relevance at various stages of carcinogenesis the regulation of AHH and UDPGT was also studied in several transformed epithelial cell lines in comparison with primary cultures of keratinocytes.

MATERIALS AND METHODS

3-Hydroxy-BP and BP-7,8-dihydrodiol were obtained from the Chemical Carcinogen Reference Standard Repository, NIH, Bethesda, MD.

Sources of whole skin and skin cells. Whole skin was obtained from adult female C57BL/6 mice and neonatal C3H mice. The following cultured skin cells were used: Keratinocytes, harvested during the first day from primary cultures of epidermal cells isolated from neonatal C3H mouse skin. Fibroblasts, harvested from early (4th–7th) passage cultures of neonatal C3H mouse skin mesenchymal cells, essentially free of keratinocytes. Skin cells were isolated, characterized and cultivated as described earlier [21].

Transformed keratinocytes from four tumorigenic cell lines with different origins were used: (1) *HEL-30 cells*, a spontaneously transformed keratinizing cell line, originating from a long-term primary cul-

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§ Abbreviations used: UDPGT, UDP-glucuronyltransferase; AHH, arylhydrocarbon hydroxylase; BP, benzo[*a*]pyrene; BA, benz[*a*]anthracene; DMBA, 7,12-dimethylbenz[*a*]anthracene; MC, 3-methylcholanthrene.

ture of C3H neonatal keratinocytes [22]. (2) *HEL-37 cells*, a non-keratinizing variant of HEL-30 [22]. (3) *BD VII cells*, a cell line transformed *in vitro* by a single treatment of C57BL/6 mice with DMBA [22]. (4) *HD II cells*, a carcinoma cell line derived from a C3H mouse skin tumor induced by repeated application of DMBA [23].

Treatment with Aroclor 1254. Aroclor 1254 (500 mg/kg, dissolved in 0.3 ml acetone) was painted once on the shaved back skin of C57BL/6 mice. Skin and liver were examined after 1 week of treatment. Control animals were treated in the same way but received the solvent alone.

Preparation of whole skin and cell homogenates. Shaved back skin, including dermis and epidermis (2 × 4.5 cm) was taken from the area used for topical application of Aroclor 1254 and from the same skin area of controls. Adhering fat was removed from the dermal site. Then the skin was frozen in liquid nitrogen and placed in a cooled (liquid nitrogen) stainless steel mortar (internal diameter 30 mm) and powdered by 10 hammerstrokes on a well-fitted piston [24]. The powder was transferred to Eppendorf tubes, weighed and suspended in 0.25 M sucrose by sonication (2 × 2 sec).

Cells were scraped off from the culture dishes, suspended in phosphate-buffered saline, centrifuged for 10 min at 400 *g* and the pellets were stored at −80°. After thawing, the pellets were taken up in 0.25 M sucrose and suspended by sonication (2 × 2 sec).

Assays. Methods described previously were used for the determination of UDPGT activity towards 1-naphthol [25], towards 3-hydroxy-BP and BP-7,8-dihydrodiol [26], arylhydrocarbon hydroxylase [27] and protein [28]. UDPGT activity was measured in the presence of 0.025% (w/v) Brij 58, i.e. 0.25 mg Brij 58/mg protein, which led to optimal activation (1.5-fold). DNA from skin and cell homogenates was extracted using a modified version [29] of the Schmidt–Tannhauser procedure [30] and determined by the diphenylamine method [31]. Cell counts were estimated from eight tissue sections (300 × 7 μm) which were stained with hematoxylin and eosin. Each slide contained 250–300 cell nuclei.

RESULTS AND DISCUSSION

Quantification of drug metabolizing enzymes in isolated skin fractions is hampered by the large variability in the yield of different fractions. In pilot experiments we observed that UDPGT activity was lost to a considerable extent by centrifugation at low gravity (500 g). Therefore in the present study enzyme activities were studied in the unfractionated skin homogenate and measurements are based on tissue wet weight.

UDPGT activity was found to be high in mouse skin (Table 1), confirming earlier observations with rat skin [26]. Therefore, UDPGT may efficiently inactivate BP phenols in skin. For example, in the AHH assay 3-hydroxy-BP is formed at a rate of about 0.06 nmole/min/g tissue whereas UDPGT activity towards 3-hydroxy-BP was found to be 0.37 nmole/min/g tissue. Topical application of Aroclor 1254 induced UDPGT activities towards 1-naphthol, 3-hydroxy-BP and BP-7,8-dihydrodiol (3-fold) and AHH activity (15-fold). Inducibility of the glucuronidation of 3-hydroxy-BP and 1-naphthol by MC-type inducers suggests the presence of a particular isoenzyme of UDPGT in mouse skin, operationally termed $UDPGT_1$ or MC-inducible phenol UDPGT [32, 33]. This isoenzyme may play a special role in the detoxication of aromatic compounds, particularly in preventing toxic quinone/quinol redox cycles [34]. It has been shown to be almost ubiquitously distributed in different organs [26].

In addition to its effects on skin, topical application of Aroclor 1254 induced drug metabolizing enzymes in liver and led to a large hypertrophy in this organ. Liver weight increased from 0.9 ± 0.2 g in untreated controls to 2.1 ± 0.7 g after topical application of Aroclor 1254. The latter finding demonstrates the rapid absorption and distribution of the mixture of polychlorinated biphenyls.

For lack of a single satisfactory comparative parameter it is difficult to express enzyme activities in skin. Therefore several parameters were measured to be able to express enzyme activity in different ways and to allow a comparison of results obtained in different laboratories (Table 2). Our estimate of

Table 1. Induction of arylhydrocarbon hydroxylase and UDP-glucuronyltransferase by Aroclor 1254 in skin and liver of C57BL/6 mice

Organ and enzyme	Enzyme activity (nmole/min/g tissue) Controls	Aroclor 1254*
Skin		
AHH	0.06 ± 0.01†	0.91 ± 0.22 (15)
UDPGT (1-naphthol)	26 ± 4	72 ± 13 (3)
UDPGT (3-OH-BP)	0.37 ± 0.05	1.1 ± 0.15 (3)
UDPGT (BP-7,8-dihydrodiol)	0.12 ± 0.15	0.30 ± 0.05 (3)
Liver		
AHH	35 ± 7	289 ± 74 (8)
UDPGT (1-naphthol)	1632 ± 358	3369 ± 599 (2)
UDPGT (3-OH-BP)	65 ± 25	n.d.
UDPGT (BP-7,8-dihydrodiol)	5.1 ± 0.2	n.d.

* Topical application to the skin as described in Materials and Methods.
† The means ± S.D. of four experiments are listed.
Numbers in parentheses represent -fold increase over controls. n.d., not determined.

Table 2. Parameters used as a basis to express enzyme activity in skin and primary cultures of skin cells

Parameter	Parameter/g tissue wet weight
DNA (neonatal)	3.7 ± 0.7 mg*
DNA (adult)	1.4 ± 0.3 mg
Protein	169 ± 14 mg†
Cell number	635 ± 50 × 10^6‡
Area	46 ± 7 cm^2†

* The means ± S.D. of four determinations are listed.
† Not significantly different in neonatal and adult skin.
‡ Only determined in adult skin.

the cell number (635 × 10^6 cells/g tissue wet weight) is close to that published previously (574 × 10^6 cells/g tissue wet weight; [35]). About 35, 23 and 42% of cell nuclei were found in hair follicles, in the epidermis and dermis, respectively. Based on tissue wet weight basal enzyme activity of AHH and UDPGT (1-naphthol as substrate) did not differ significantly in adult skin of several mouse strains such as C57BL/6, DBA/2 and MNRI (not shown).

For comparison of whole skin with cultured skin cells enzyme activities were based on mg DNA. Neonatal skin of C3H mice had to be used for this purpose since the cultured cells were derived from neonatal skin of this strain. The content of DNA in neonatal skin was much higher than that found in adult skin. As shown in Table 3, UDPGT activity towards 1-naphthol was high in both keratinocytes and fibroblasts and was comparable to that found in neonatal skin. In contrast, AHH activity was higher in cultured keratinocytes than in skin. AHH activity in keratinocytes was further inducible (3-fold) by BA or DMBA (Table 4). The reason for increased AHH activity in cultured keratinocytes is unclear. The observation may be related to findings of Hosomi *et al.* in keratinocyte cultures from several "responsive" and "non-responsive" mouse strains [12]. Whereas in skin *in vivo* AHH activity could only be induced in the responsive strains in keratinocyte cultures, AHH activity could be induced in cells from both non-responsive and responsive strains. According to Hosomi *et al.*, the discrepant findings *in vivo* and *in vitro* may suggest that in non-responsive mice the genes involved in AHH induction are present but suppressed by some unknown host factors in skin [12].

Table 3. UDPGT (1-naphthol) and AHH activities in cultured skin cells and in skin tissue

Cell type, tissue	UDPGT (nmole/min/mg DNA)	AHH (nmole/min/mg DNA)
Keratinocyte	4.7 ± 2.7*	0.24 ± 0.05
Fibroblasts	7.9 ± 3.3	0.02
HEL-30 cells	22.1 ± 6.2	0.02
HEL-37 cells	17.6†	0.01
BD VII cells	29.4	n.d.
HD II cells	6.6	n.d.
Skin (neonatal)	7.9 ± 0.8	0.018 ± 0.002
Skin (adult)	19 ± 3	0.026 ± 0.007

* The means ± S.D. of three to four determinations are listed.
† Means of duplicate determinations.
n.d., not determined.

UDPGT activity in transformed epithelial cell lines (HEL-30, HEL-37 and BD VII) (with the exception of the carcinoma cell line HD II) appear to be significantly higher than in keratinocytes (Table 3). In contrast, AHH activity was low in the transformed cells. However, AHH activity was still inducible by BA or DMBA (3- to 4-fold, Table 4). In comparison with nontransformed cells basal UDPGT activity in three transformed epithelial cell lines was much higher (4- to 6-fold). In contrast, AHH activity was reduced to less than 10%. This alteration in the pattern of the two functionally related phase I and II enzymes is similar to that observed after initiation of hepatocarcinogenesis in rats [14–19, 36]. In liver nodules several cytochrome P-450-dependent reactions were found to be decreased [14, 17] whereas conjugating enzymes such as UDPGTs [17, 18] and GSH-transferases [36] were enhanced. This altered pattern of drug metabolizing enzymes is consistent with increased toxin resistance of transformed cells. Upon continuous exposure to toxins transformed cells would gain a growth advantage over non-transformed cells since primarily the latter would suffer from the mitoinhibitory effect of carcinogens. Hence this toxin resistance phenotype may select for transformed cells and may thus be important for the evolution of cancer cells [20]. Increased UDPGT and decreased AHH activity in skin were also observed after long-term irradiation of hairless mice with u.v.-light (Lilienblum, Berger and Bock, unpublished results).

The present study provides methods to investigate

Table 4. Influence of BA and DMBA on AHH activity in normal and transformed skin cells*

Cell type	AHH activity (pmole/min/mg DNA) Control	BA	DMBA
Keratinocytes	221 ± 48†	709 ± 86	581 ± 176
HEL-30 cells	14 ± 6	56 ± 3	35 ± 7
Fibroblasts	9‡	31	16

* Cultures were treated for 24 hr with BA (5×10^{-6} M) or DMBA (8×10^{-7} M).
† Data represent the means ± S.D. of three to four experiments.
‡ Means of duplicate determinations.

induction of UDPGT and AHH quantitatively in mouse skin. The methods facilitate a comparison of the regulation of drug metabolizing enzymes in skin *in vivo* and in skin cell cultures. The high AHH activity in cultured keratinocytes in comparison with whole skin and the altered pattern of drug metabolizing enzymes (decreased drug oxidation and increased glucuronidation) in transformed epithelial cell lines requires further study.

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