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**Running Title:** Cutaneous *CYP1B1* transcriptional induction by UV-B

**Increase of *CYP1B1* transcription in human keratinocytes and HaCaT cells  
after UV-B exposure**

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Nonmelanoma skin cancers represent the most common malignant neoplasms in humans. UV-B play a major role in the etiology of these tumors, but exposure to environmental procarcinogens is also involved. CYP catalyzes numerous chemical carcinogen bioactivations and effects of UV-B on their expression are poorly understood. The aim of this study was to explore the molecular events involved in the induction of CYP1B1, a major cutaneous CYP, by UV-B. Our results demonstrated that unique UV-B exposure (20 mJ/cm<sup>2</sup>) increases human CYP1B1 transcript in primary keratinocytes and HaCaT cell cultures. Among 20 human samples studied, we observed a large inter-individual variability of CYP1B1 mRNA induction (1.1 to 4.5-fold). Pretreatment with an antioxidant, N-acetylcysteine, repressed CYP1B1 increase, suggesting the involvement of UV-B photoproducts.  $\alpha$ -Amanitin inhibition studies and CAT assays demonstrated that CYP1B1 mRNA induction is associated with a transcriptional activation of its expression.  $\alpha$ -Naphthoflavone inhibition studies and CAT assays performed after directed mutagenesis of XRE sites showed the involvement of Ah receptor. Taken together, these data demonstrated that UV-B induces *CYP1B1* gene expression after an activation of its transcription, which involves Ah receptor.

**Key Words:** CYP1B1, human keratinocytes, UV-B, Ah receptor

Nonmelanoma skin cancers (NMSC), including basal cell carcinomas and squamous cell carcinomas, represent the most common malignant neoplasms in humans. The most important factor contributing to the development of skin cancers is chronic exposure to UV radiation in sunlight, notably UV-B (Grossman and Leffell, 1987), which is directly genotoxic through a physical mechanism. However, Yuspa has suggested that xenobiotic metabolizing enzymes, especially cytochromes P450 (CYP), could also be involved through the bioactivation of environmental procarcinogens (Yuspa, 1986). This hypothesis was further supported by the work of Kanjilal *et al.* (Kanjilal *et al.*, 1995) who studied p53 mutations in tumors and adjacent nonmalignant skin samples from eight patients with NMSC and exposed to carcinogens from industrial or agricultural sources. It is known that UV-B exposure induces C→T and CC→TT transitions at dipyrimidine sequences. In their study, the mutations consisted of C→T transitions at dipyrimidine sequence, T→C transitions, and G→T transversions, suggesting that other carcinogens may act along with UV-B radiation in the development of NMSC.

Procarcinogens are chemically inert and require bioactivation to exert their genotoxic effects. CYP are widely involved in these bioactivation processes (Gonzalez and Gelboin, 1994). Among them, CYP1B1 appears to play a key role. This isozyme catalyzes the bioactivation of environmental compounds such as polycyclic aromatic hydrocarbons (PAH) and arylamines (Shimada *et al.*, 1996; Shimada *et al.*, 1997). It is also involved in the bioactivation of 17-βestradiol to 4-hydroxyestradiol, which undergoes metabolic redox cycling and induces mutagenic free radicals (Liehr *et al.*, 1995). Moreover, CYP1B1 has been found to be expressed at a high level in a wide range of human cancers of different histogenetic types, including nonmelanoma skin cancers (Murray *et al.*, 1997).

The effect of UV-B irradiation on cutaneous CYP expression is poorly known. Previous studies have demonstrated that exposure to UV-B increases various CYP1A1-related

enzymatic activities in rodent skin (Mukhtar *et al.*, 1986; Goerz *et al.*, 1996). Similar results have been obtained using human keratinocytes from postmortem skin samples which were either sun-exposed, or sun-protected (Hirel *et al.*, 1996).

*CYP1* expression is mainly regulated by Ah receptor (AhR). Briefly, ligand such as PAH and dioxins induces *CYP1* genes through binding to AhR, translocation of the ligand-bound AhR into the nucleus, and association of the AhR with AhR nuclear translocator (Arnt). The AhR-Arnt complex then binds to xenobiotic responsive element (XRE) and turns on *CYP1* gene expression (Hankinson, 1995). UV-B-induced formylated derivatives of tryptophan have been described as being potent AhR agonists which suggests that they are at least in part involved in CYP1A1 induction by UV-B exposure (Helferich and Denison, 1991; Wei *et al.*, 1998; Wei *et al.*, 1999). It has been recently observed that UV-B exposure can induce CYP1A1 and CYP1B1 expression in human epidermis at protein and mRNA levels (Katiyar *et al.*, 2000). However, no data are available concerning the role of UV-B photoproducts and mechanisms involved in CYP1B1 induction.

The aim of our study was to assess the molecular events involved in *CYP1B1* induction by UV-B.

## MATERIALS AND METHODS

### *Materials*

Materials and their sources were as follows: DMEM, phosphate buffered saline (PBS), and HAM'S F12 (Eurobio, Les Ulis, France); fetal calf serum, fungizone, L-glutamine, and sodium pyruvate (Life Technologies, Cergy Pontoise, France); N-acetylcysteine (NAC),  $\alpha$ -amanitin,  $\alpha$ -naphthoflavone ( $\alpha$ NF), bovine serum albumin (BSA), cholera toxin, hydrocortisone, insulin, 3-methylcholanthrene (3MC), penicillin, streptomycin, triiodothyronine, and tryptophan (SIGMA, St. Quentin Fallavier, France); epidermal growth factor (Roche Molecular Biochemicals, Meylan, France); collagen from rat tail (Institut J. Boy, Reims, France); plastic cell culture dishes (Sarstedt, Mercey le Grand, France).

### *Keratinocyte cultures*

Human skin was obtained, under strict ethical conditions from women undergoing surgical resection for breast hypertrophy and who had given their informed consent. Keratinocytes were extracted as previously described (Rheinwald and Green, 1975). Cells were plated on culture dishes coated with collagen and were routinely cultured in medium containing 65% of DMEM, 25% of HAM'S F12, 10% of fetal calf serum, 5.4  $\mu$ g/ml of insulin, 9.13 ng/ml of cholera toxin, 1.5 ng/ml of triiodothyronine, 0.43 ng/ml of hydrocortisone, 10.8 ng/ml of epidermal growth factor, 100U/ml of penicillin and 100  $\mu$ g/ml of streptomycin.

Each experiment was performed after only one passage. We had to use different preparations from different donors because the size of skin samples did not provide enough material to perform all of the experiments with the same sample.

### *HaCaT cell cultures*

HaCaT cells were kindly provided by Dr. Catroux (L'Oreal Recherche) and used between passage 51 and 54. They were cultured in DMEM containing 10% fetal calf serum supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), amphotericine-B (2.5 µg/ml), penicillin (50 U/ml), and streptomycin (50 µg/ml).

### *Exposure to UV-B*

The UV light source was a Waldmann DHL-111 lamp (Waldmann, Villingen-Schwenningen, Germany) that emitted UV light between 280 and 360 nm (peak 313 nm). The energy was measured with a Waldmann UV-meter (model n° 585.100, Waldmann). At confluence, keratinocytes or HaCaT cells were washed twice with PBS, exposed in 1 ml PBS to UV-B (20 mJ/cm<sup>2</sup>), washed three times in PBS, and cultured in medium for 0-24 h for determination of the peak of CYP1B1 mRNA. For the other experiments, human keratinocytes and HaCaT cells were cultured in medium after UV-B exposure for 5h and 6h respectively. A dose of 20 mJ/cm<sup>2</sup> has been shown to not compromise cell survival (Garmyn and Degreef, 1997).

### *Other treatments*

In order to check the possible role of UV-B photoproducts in the induction of CYP1B1 transcript by UV-B exposure, keratinocytes or HaCaT were pretreated with NAC 1 mM for 3 days prior to exposition and until RNA extraction. Indeed, NAC 1 mM was described to inhibit oxidative stress induced by UV-B in human keratinocyte cultures (Garmyn and



Degreef, 1997). In another experiment, HaCaT cells were grown in medium supplemented with tryptophan aqueous solutions exposed or unexposed to UV. Briefly, a 10 mg/ml aqueous solution of tryptophan (TRP) was exposed to unfiltered high pressure mercury lamp at distance of ~15 cm, for 4 h to generate photoproducts and obtain TRP-UV solution. Then, HaCaT cells were treated for 24 h with TRP, or TRP-UV aqueous solution (1 µl/ml of medium).

In order to study the implication of AhR in the CYP1B1 induction by UV-B, keratinocytes or HaCaT cells were treated as previously described with a type II RNA polymerase inhibitor  $\alpha$ -amanitin at 2 µg/ml (Elferink and Reiners, 1996), or an AhR antagonist  $\alpha$ NF 1 µM (Merchant *et al.*, 1992), or an AhR agonist 3MC 5 µM.

#### *Determination of CYP1B1 mRNA level by RT-PCR analysis*

Total cellular RNA was isolated from keratinocyte cell cultures using RNAXEL<sup>®</sup> RNA isolation kit (Eurobio, Les Ulis, France). One µg of total RNA was reverse-transcribed in 30 µl using GibcoBRL M-MLV reverse-transcriptase (Life Technologies, Cergy Pontoise, France) in its own buffer and random primers at 37°C for 1 h. The cDNA product (20 ng) was amplified in 25 µl volume using 200 µM of each of the four desoxyribonucleoside triphosphates, 125 ng of each primer (Table 1), 1.5 mM or 1.6 mM of MgCl<sub>2</sub> for  $\beta$ 2-microglobulin and *CYP1B1* respectively, 0.3 unit of Taq polymerase in its own buffer (EUROBIOTAQII<sup>®</sup> ADN polymerase, EUROBIO, Les Ulis, France), and for *CYP1B1* 0.2 mg/ml of BSA. PCR was performed using the GeneAmp<sup>®</sup> PCR System 2400 (Perkin Elmer, Branchburg, U.S.A.) and carried out as follows:

- for *CYP1B1*: 3 min. at 93°C, 1 min. at 60°C, 1 min. at 72°C, and 34-fold 30 sec. at 91°C, 30 sec. at 54°C, 1 min. at 72°C.

- for  $\beta$ 2- microglobulin: 3 min. at 93°C, 1 min. at 53°C, 1 min. at 72°C, and 25-fold 30 sec. at 91°C, 30 sec. at 53°C, 1 min. at 72°C.

The specificity of for  $\beta$ 2-microglobulin primers has been already studied by El Kyari *et al.* (El Kyari *et al.*, 1997). The 5' primer of CYP1B1 was selected by our laboratory and was localized on the 3' end of exon 2 and the 5' flanking region of exon 3. The 3' primer of CYP1B1 was located within exon 3. After amplification, the PCR reaction product was electrophorized on a 2% agarose gel. The gel was then stained with ethidium bromide and the image was digitalized using the Appligene Imager (Appligene, France). The negative controls omitted the reverse-transcription reaction, or the cDNA product (data not shown). The yield of cytochrome P450 isoforms was normalized to  $\beta$ 2-microglobulin after quantitative estimation using NIH Image software (Bethesda, U.S.A.). PCR analyses were performed in triplicate.

In order to check the semiquantitative response of our RT-PCR experiment, cDNA from K59 sample with high CYP1B1 expression was diluted 1-20 fold. For each dilution, 9 independent PCR experiments were performed and CYP1B1 relative expression levels were evaluated (data not shown). The absence of statistical differences (set at  $p < 0.05$ ) between groups of dilution was assessed by the Anova test, and the Newman-Keuls test was used for group-by-group comparisons. No statistical difference was observed between different dilution groups.

#### *CAT assay experiments and oligonucleotide-directed PCR mutagenesis*

Human keratinocytes were grown to 60-70% confluence before transfection. Transient transfection was performed by lipofection in a serum and antibiotic-free medium (Lipofectin<sup>®</sup> reagent, Life Technologies, Cergy Pontoise, France) with 2  $\mu$ g of pCAT-(wtXRE promoter) from Pr. W.F. Greenlee laboratory and 0.5  $\mu$ g  $\beta$ -galactosidase expression vector kindly

provided by Pr. Champion (UMR CNRS 6032, Marseille, France). pCAT-(wtXRE promoter) included the fragment -1022/-835 of the 5' flanking region of *CYP1B1* gene, with the 3 active XRE sites (Tang *et al.*, 1996). After incubation for 24h, the lipofection solution was replaced with normal growth medium and incubated for 48h. Transfectants were exposed or not exposed to UV-B, in the absence or presence of  $\alpha$ -NF as described above. After incubation for 24h, the CAT protein level was determined by means of the CAT Elisa Kit (Roche Molecular Biochemicals, Meylan, France). Results were expressed as relative CAT expression which corresponds to pmol of CAT protein per mg of total proteins normalized by  $\beta$ -galactosidase activity. Total protein amount was determined by the Bradford method (Bradford, 1976). All CAT assay experiments were performed in triplicate.

In order to confirm the involvement of AhR, HaCaT cells were transfected with pCAT-(wtXRE promoter) or pCAT-( $\Delta$ XRE promoter). In the latter construction, the three XRE sites were mutated by oligonucleotide-directed PCR mutagenesis (QuickChange® site-directed mutagenesis kit, Stratagene, Amsterdam, Netherlands). The complementary mutagenic primers are as follows (cis acting element in underlined boldface): XRE1 (-993 to -989) 5'-GGTGGCGGCCGGCACCC**GGGGC**CAAGGGTGGTGGTGG-3' and 5'-CCACCA-CCACCCTTGG**CCCCG**GGGTGCCGGCCGCCACC-3'; XRE2 (-944 to -940) 5'-GGCC-GCCGCCTCC**GCTGA**TCAGGTGCCGTG-3' and 5'-CACGGCACCTGAT**TCAGC**GGAG-GCGGCGGCC-3'; XRE3 (-857 to -853) 5'-CCAGAAGCGGCC**CGGGC**AAAGCCCA-GCTCC-3' and 5'-GGAGCTGGGCTT**TGCC**CGGGCCGCTTCTGG-3'. HaCaT cells were grown to 50-60% confluence before transfection. Transient transfection was performed by lipofection in a serum and antibiotic-free medium (Superfect® reagent, Qiagen, Courtaboeuf, France) with 1.5  $\mu$ g of either pCAT-(wtXRE promoter) or pCAT-( $\Delta$ XRE promoter) and 0.5  $\mu$ g  $\beta$ -galactosidase expression vector. After incubation for 24h, the lipofection solution was replaced with normal growth medium and incubated for 48h. Transfectants were exposed or

not exposed to UV-B. After incubation for 24h, the CAT protein level was determined as described above. All CAT assay experiments were performed 5 times.

## RESULTS

### *Kinetic of CYP1B1 transcript induction by UV-B exposure (Figure 1)*

Figure 1A displays CYP1B1 transcript induction by UV-B performed on samples from 5 different patients. The peak of CYP1B1 mRNA was observed between 4-6 h after the treatment, depending on the sample used. In HaCaT cells (n= 3), the peak of transcript was observed 6 h after the treatment (Fig. 1B).

### *Inter-individual variability of CYP1B1 transcript induction by UV-B exposure (Figure 2)*

As shown in Figure 2, a large inter-individual variability for CYP1B1 mRNA induction by UV-B exposure was observed among samples from 20 different donors. The induction ranged from 1.1- to 4.5-fold, depending on the sample studied.

### *Involvement of UV-B photoproducts in the CYP1B1 transcript induction by UV-B exposure (Figure 3)*

In order to study the involvement of UV-B photoproducts, cells were exposed to UV-B with or without a pretreatment with a potent anti-oxidant, NAC. Experiments in human keratinocytes were performed on two samples, K6 and K37, which exhibited low and high inducibility after UV-B treatment. As shown in figure 3A, the CYP1B1 transcript induction was suppressed by NAC in K6 sample and reduced by 67% in K37 sample. Similarly, CYP1B1 mRNA induction was suppressed in HaCaT cells by NAC treatment (Fig. 3B).

It has been suggested that CYP1A1 induction by UV-B is mediated at least in part by UV-induced formylated derivatives of tryptophan which are potent AhR agonists (Helferich

and Denison, 1991; Wei *et al.*, 1998; Wei *et al.*, 1999). Therefore, unexposed cells were treated with either unexposed or exposed tryptophan solutions (TRP and TRP-UV respectively). HaCaT treatment by TRP-UV induced CYP1B1 mRNA 1.7-fold, while TRP had no effect.

Taken together, these data strongly suggested the involvement of UV-B photoproducts in the CYP1B1 induction by UV-B exposure.

*Transcriptional activation of CYP1B1 gene expression after UV-B exposure through AhR pathway (Figures 4 and 5)*

All further experiments in human keratinocytes were performed on K18 and K19 samples which provided enough material to perform the study.

In order to investigate an activation of *CYP1B1* gene transcription by UV-B, keratinocytes and HaCaT cells were pretreated with  $\alpha$ -amanitin (2  $\mu$ g/ml), a potent inhibitor of type II RNA polymerase. UV-B induction of CYP1B1 mRNA in K18 sample was strongly reduced by  $\alpha$ -amanitin (Fig. 4A) and suppressed in HaCaT cells (Fig. 4B). This transcriptional activation of *CYP1B1* gene was confirmed in keratinocytes and HaCaT cells, transiently transfected with pCAT-(wtXRE promoter) and exposed to UV-B. CAT expression was induced 1.7-fold in K18 sample (Fig. 5A) and HaCaT cells (Fig. 5B) after UV-B exposure. Similar results were obtained with K19 sample with a 2.2-fold induction of *CYP1B1* transcription (data not shown).

In order to investigate the involvement of AhR in UV-B induced *CYP1B1* transcriptional activation, K18 keratinocytes and HaCaT cells were treated with  $\alpha$ NF (1  $\mu$ M), an antagonist of AhR. CYP1B1 mRNA induction was strongly reduced by  $\alpha$ NF in K18 sample (Fig. 4A) and suppressed in HaCaT cells (Fig. 4B). Similarly, in K18 keratinocytes transfected with pCAT-(wtXRE promoter), the transcriptional activation of *CYP1B1* by UV-B

or 3MC treatment were repressed by  $\alpha$ NF (Fig. 5A). These data suggested that AhR was involved in CYP1B1 induction by UV-B exposure. In addition, HaCaT cells were transiently transfected by pCAT-( $\Delta$ XRE promoter), where the three XRE sites (where binds AhR) were mutated. As shown in figure 5B, the mutation of XRE sites suppressed the increase of CAT expression induced by UV-B exposure, strengthening the involvement of AhR pathway.

## DISCUSSION

Our results demonstrated that unique UV-B exposure induces *CYP1B1* gene expression in human keratinocytes and in human keratinocyte cell line HaCaT, after a transcriptional activation which involves AhR.

In 20 individual samples, we observed a large inter-individual variability of *CYP1B1* inducibility by UV-B exposure. The variability observed between individuals (28% to 143%) was greater than coefficient of variation of RT-PCR in the same subject (about 15%). The factor of *CYP1B1* mRNA induction by UV-B was between 1.2 and 4.5. This can be compared to unpublished data from our laboratory, showing that 3MC (5  $\mu$ M) induced 1.5 to 3.4 fold *CYP1B1* mRNA in human keratinocytes. Inter-individual variability of *CYP1A1* induction has been largely described and was associated with a genetic polymorphism of induction (Catteau *et al.*, 1995). The genetic polymorphism of *CYP1A1* induction has been recently associated in part with G1721A allelic variant of AhR in caucasian population (Smart and Daly, 2000). In addition, different genetic polymorphisms in exons 2 and 3 of human *CYP1B1* gene inducing some alterations in catalytic function towards procarcinogens and steroid hormones have been already described (Shimada *et al.*, 1999). The inter-individual variability of *CYP1B1* induction by UV-B could be explained by genetic polymorphisms of *CYP1B1* induction and/or AhR, but further experiments with larger cohorts are required. Individuals with high *CYP1B1* inducibility by UV-B could present a higher risk of cancers (including NMSC) due to environmental carcinogens.

Pretreatment of keratinocytes or HaCaT cells with a well-known antioxidant compound, NAC, repressed the induction of *CYP1B1* mRNA by UV-B. This result suggests the involvement of UV-B photoproducts. UV-induced formylated derivatives of tryptophan are potent AhR agonists of AhR (Helferich and Denison, 1991; Wei *et al.*, 1998; Wei *et al.*,



1999) and could be involved at least in part in CYP1B1 induction by UV-B, along with probably other photoproducts. Therefore, HaCaT cells were treated with tryptophan solution or tryptophan solution exposed to UV. Tryptophan exposed solution induced 1.7-fold CYP1B1 transcript, while unexposed solution had no effect.

Using the pCAT-(wtXRE promoter), we observed an induction of CAT expression in human keratinocytes and HaCaT cells. These results are in agreement with the inhibition of CYP1B1 mRNA induction by UV-B exposure, after a pretreatment of cells with  $\alpha$ -amanitin. Therefore, UV-B induced *CYP1B1* expression at a transcriptional level.

*CYP1B1* gene expression is mainly regulated by AhR. A pretreatment of human keratinocytes and HaCaT cells with  $\alpha$ NF, a potent AhR antagonist, repressed CYP1B1 transcript induction by UV-B, suggesting the involvement of AhR pathway.

In order to confirm the role of AhR, the three XRE sites located in pCAT-(wtXRE promoter) were mutated to obtain pCAT-( $\Delta$ XRE promoter). When HaCaT cells were transiently transfected with the latter construction, we did not observe any induction of CAT expression after UV-B exposure.

Taken together, these results demonstrate that UV-B induces *CYP1B1* expression after transcriptional activation. This activation involved at least UV-B photoproducts and AhR.

CYP1B1 was found to have higher catalytic activities than CYP1A1 for the activation of numerous PAH (Shimada, 1996). It has been recently demonstrated that Cyp1b1, but not Cyp1a1, is responsible for 7,12-dimethylbenz[a]anthracene-induced lymphoma in mice (Buters *et al.*, 1999). CYP1B1 catalyzes the bioactivation of heterocyclic and aryl amines, including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) which would appear to be one of the most important heterocyclic amines consumed in diet (Shimada, 1996; Crofts *et al.*, 1997). CYP1B1 also catalyzes the bioactivation of 17- $\beta$ -estradiol into 4-hydroxyestradiol which generates mutagenic free radicals (Liehr *et al.*, 1995). These data suggest that the

induction of cutaneous *CYP1B1* by UV-B could act along with UV radiation in the development of NMSC, notably in workers exposed to PAH or to other procarcinogens bioactivated by CYP1B1. Moreover, it has been demonstrated in rodents that UV-B exposure is able to increase CYP1A1 enzymatic activity in non-cutaneous tissues, such as liver (Mukhtar *et al.*, 1986; Goerz *et al.*, 1996). A similar phenomenon could also occur with CYP1B1, also increasing cancer risk in various tissues.

In conclusion, our results demonstrate that UV-B exposure induces *CYP1B1* transcription. This phenomenon involved at least UV-B photoproducts, such as formylated derivatives of tryptophan and AhR pathway. Moreover, our data demonstrated a large inter-individual variation of CYP1B1 induction. Thus, CYP1B1 could be involved in the development of nonmelanoma skin cancers, notably in workers with high CYP1B1 inducibility and coexposed to PAH or other procarcinogens in their industrial workplaces.

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## REFERENCES

- Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
- Buters, J. T., Sakai, S., Richter, T., Pineau, T., Alexander, D. L., Savas, U., Doehmer, J., Ward, J. M., Jefcoate, C. R., and Gonzalez, F. J. (1999). Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[a]anthracene-induced lymphomas. *Proc. Natl. Acad. Sci. USA* **96**, 1977-1982.
- Catteau, A., Douriez, E., Beaune, P., Poisson, N., Bonaiti-Pellie, C., and Laurent, P. (1995). Genetic polymorphism of induction of CYP1A1 (EROD) activity. *Pharmacogenetics* **5**, 110-119.
- Crofts, F. G., Strickland, P. T., Hayes, C. L., and Sutter, T. R. (1997). Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) by human cytochrome P450 1B1. *Carcinogenesis* **18**, 1793-1798.
- El Khyari, S., Bourgarel, V., Barra, Y., Braguer, D., Briand, C. (1997). Pretreatment by tubulin agents decreases c-myc induction in human colon carcinoma cell line HT29-D4. *Biochem. Biophys. Res. Commun.* **3**, 751-754, doi:10.1006/bbrc.1997.6187.
- Elferink, C. J. and Reiners, J. J. Jr. (1996). Quantitative RT-PCR on CYP1A1 heterogeneous nuclear RNA: a surrogate for the in vitro transcription run-on assay. *Biotechniques* **20**, 470-477.
- Garmyn, M. and Degreef, H. (1997). Suppression of UVB-induced c-fos and c-jun expression in human keratinocytes by N-acetylcysteine. *J. Photochem. Photobiol.* **37**, 125-130.
- Goerz, G., Barnstorf, W., Winnekendonk, G., Bolsen, K., Fritsch, C., Kalka, K., and Tsambaos, D. (1996). Influence of UVA and UVB irradiation on hepatic and cutaneous P450 isoenzymes. *Arch Dermatol. Res.* **289**, 46-51.
- Gonzalez, F. J. and Gelboin, H. V. (1994). Role of humans cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab. Rev.* **26**, 165-183.
- Grossman, D. and Leffell, D. J. (1997). The molecular basis of nonmelanoma skin cancer. *Arch. Dermatol.* **133**, 1263-1270.
- Hankinson, O. (1995). The aryl hydrocarbon receptor complex. *Annu. Rev. Pharmacol. Toxicol.* **35**, 307-340.
- Helferich, W. G. and Denison, M. S. (1991). Ultraviolet photoproducts of tryptophan can act as dioxin agonists. *Mol. Pharmacol.* **40**, 674-678.

- Hirel, B., Watier, E., Chesne, C., Patoux-Pibouin, M., and Guillouzo, A. (1996). Culture and drug biotransformation capacity of adult human keratinocytes from post-mortem skin. *Br. J. Dermatol.* **134**, 831-836.
- Kanjilal, S., Strom, S. S., Clayman, G. L., Weber, R. S., el-Naggar, A. K., Kapur, V., Cummings, K. K., Hill, L. A., Spitz, M. R., Kripke, M. L., and Ananthaswamy, H. N. (1995). p53 mutations in nonmelanoma skin cancer of the head and neck: molecular evidence for field cancerization. *Cancer Res.* **55**, 3604-3609.
- Katiyar, S. K., Matsui, M. S., and Mukhtar, H. (2000). Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. *J. Invest. Dermatol.* **114**, 328-333.
- Liehr, J. G., Ricci, M. J., Jefcoate, C. R., Hannigan, E. V., Hokanson, J. A., and Zhu, B. T. (1995). 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: implications for the mechanism of uterine tumorigenesis. *Proc. Natl. Acad. Sci. U S A* **92**, 9220-9224.
- Merchant, M., Morrison, V., Santostefano, M., and Safe, S. (1992). Mechanism of action of aryl hydrocarbon receptor antagonists: inhibition of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced CYP1A1 gene expression. *Arch. Biochem. Biophys.* **298**, 389-394.
- Mukhtar, H., DelTito, B. J. Jr, Matgouranis, P. M., Das, M., Asokan, P., and Bickers, D. R. (1986). Additive effects of ultraviolet B and crude coal tar on cutaneous carcinogen metabolism: possible relevance to the tumorigenicity of the Goeckerman regimen. *J. Invest. Dermatol.* **87**, 348-353.
- Murray, G. I., Taylor, M. C., McFadyen, M. C., McKay, J. A., Greenlee, W. F., Burke, M. D., and Melvin, W. T. (1997). Tumor-specific expression of cytochrome P450 CYP1B1. *Cancer Res.* **57**, 3026-3031.
- Rheinwald, J. G. and Green, H. (1975). Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell* **6**, 331-343
- Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., and Sutter, T. R. (1996). Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* **56**, 2979-2984.
- Shimada, T., Gillam, E. M., Sutter, T. R., Strickland, P. T., Guengerich, F. P., and Yamazaki, H. (1997). Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metab. Dispos.* **25**, 617-622.
- Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T. R., Guengerich, F. P., Gillam, E. M., and Inoue, K. (1999). Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis* **20**, 1607-1614.
- Smart, J. and Daly, A. K. (2000). Variation in induced CYP1A1 levels: relationship to CYP1A1, Ah receptor and GSTM1 polymorphisms. *Pharmacogenetics* **10**, 11-24.

- Tang, Y. M., Wo, Y. Y. P., Stewart, J., Hawkins, A. L., Griffin, C. A., Sutter, T. R., and Greenlee, W. F. (1996). Isolation and characterization of the human cytochrome P450 CYP1B1 gene. *J. Biol. Chem.* **271**, 28324-28330.
- Wei, Y. D., Helleberg, H., Rannug, U., and Rannug, A. (1998). Rapid and transient induction of CYP1A1 gene expression in human cells by the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole. *Chem. Biol. Interact.* **110**, 39-55.
- Wei, Y. D., Rannug, U., and Rannug, A. (1999). UV-induced CYP1A1 gene expression in human cells is mediated by tryptophan. *Chem. Biol. Interact.* **118**, 127-140.
- Yuspa, S. H. (1986). Cutaneous chemical carcinogenesis. *J. Am. Acad. Dermatol.* **15**, 1031-1044.

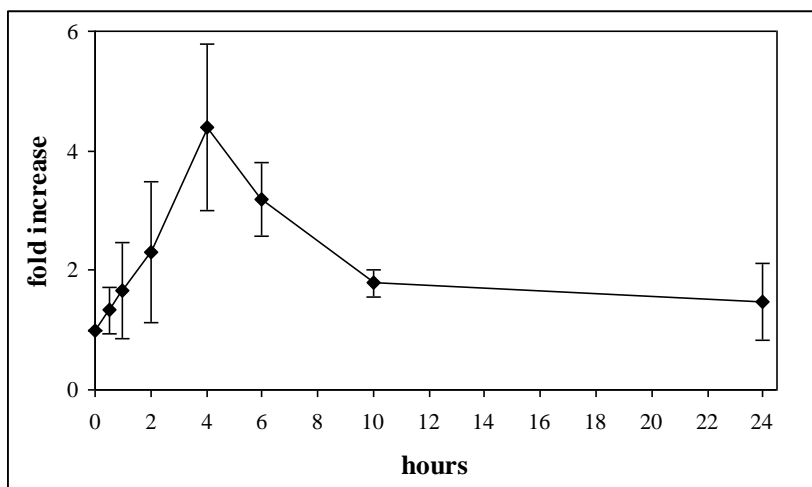
**TABLE 1****Sequences and T<sub>m</sub> values of primers used in RT-PCR experiments**

<b>Primer name</b>	<b>Sequence (5'-3')</b>	<b>T<sub>m</sub></b>
CYP1B1-S	CTTCACCAGGTATCCTGATG	58°C
CYP1B1-AS	GCAGGCTCATTGGGTTGGC	61°C
β2-Microglobulin-S	CCGACATTGAAGTTGACTTAC	58°C
β2-Microglobulin-AS	ATCTTCAAACCTCCATGATG	55°C

-S and -AS were Sense and Anti-Sense respectively

## FIGURES

A



B

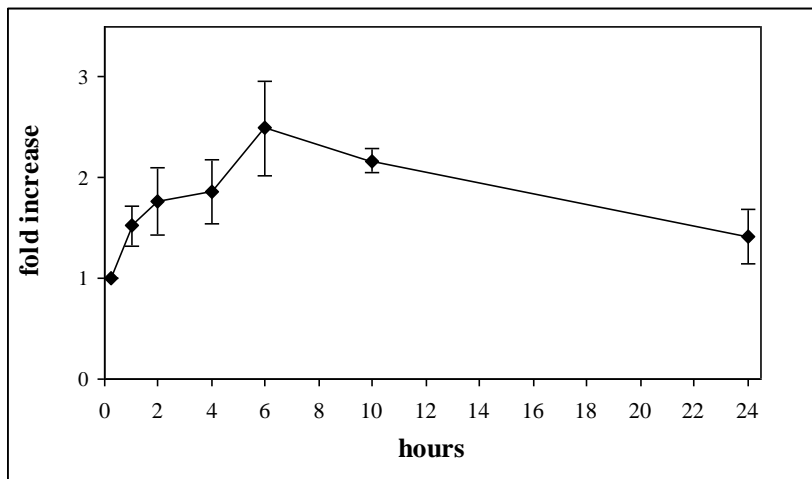
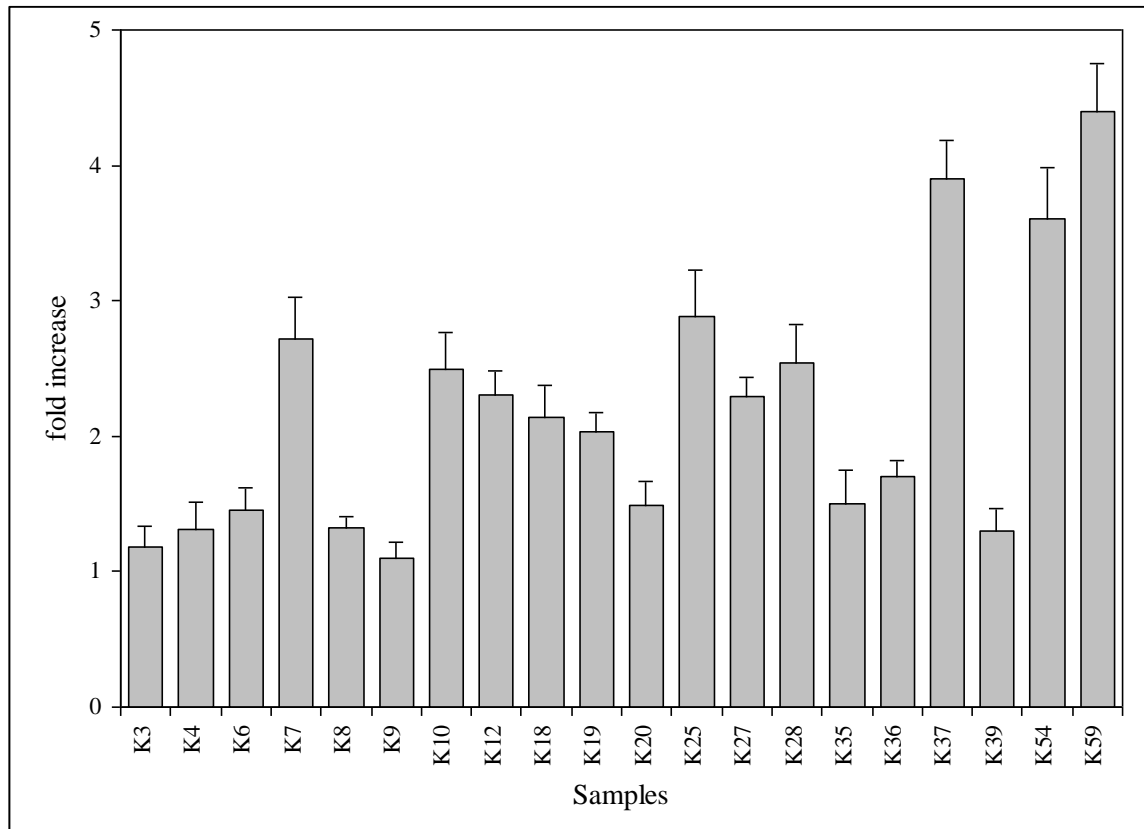
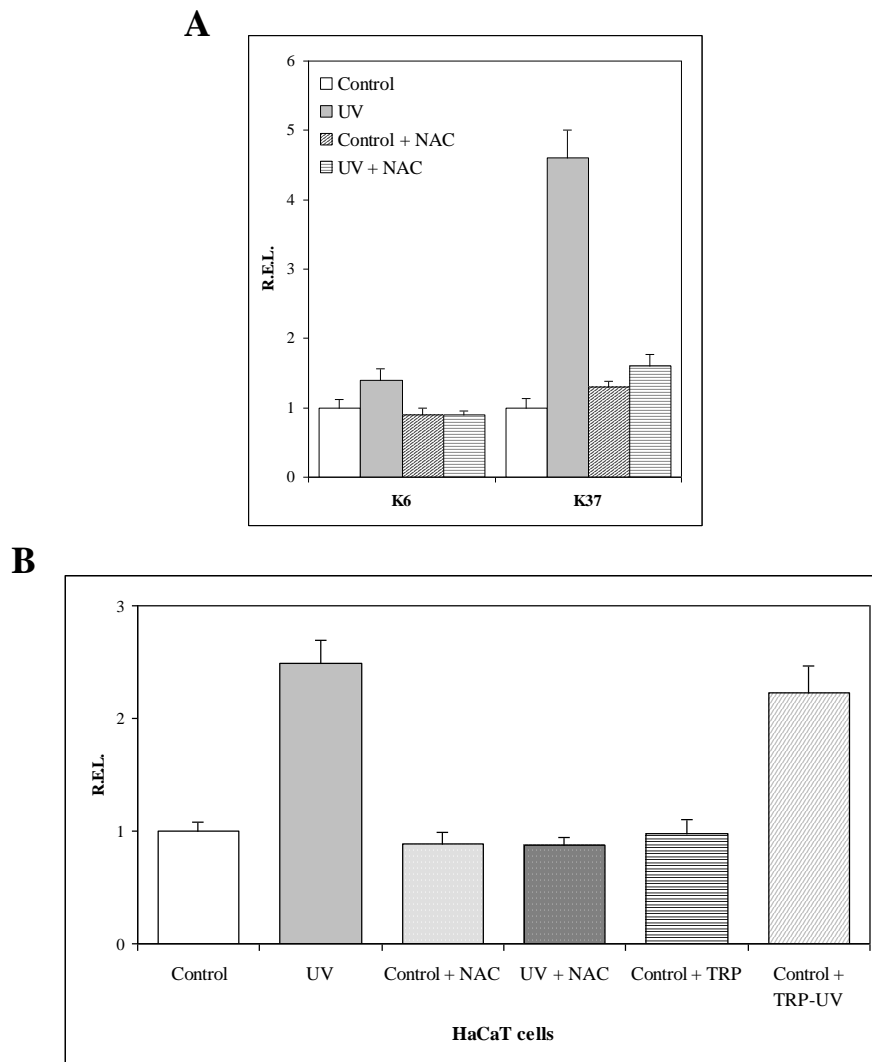
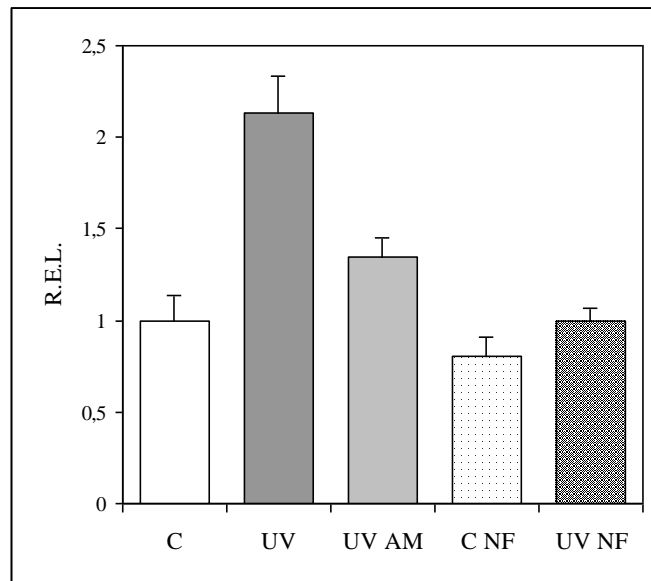
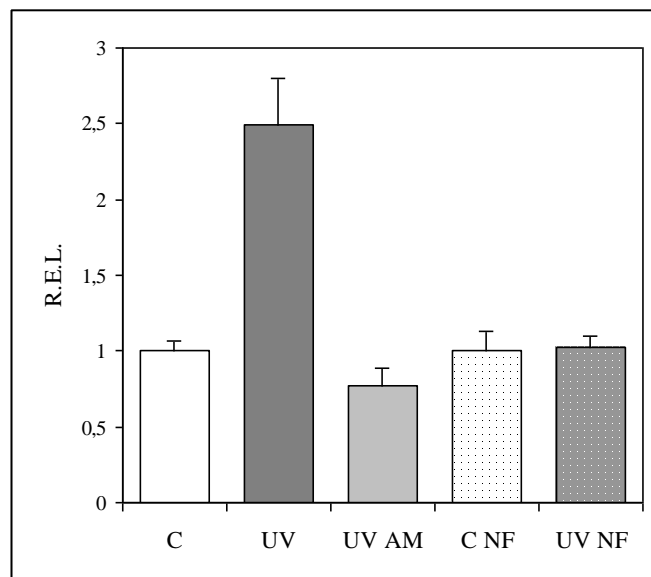


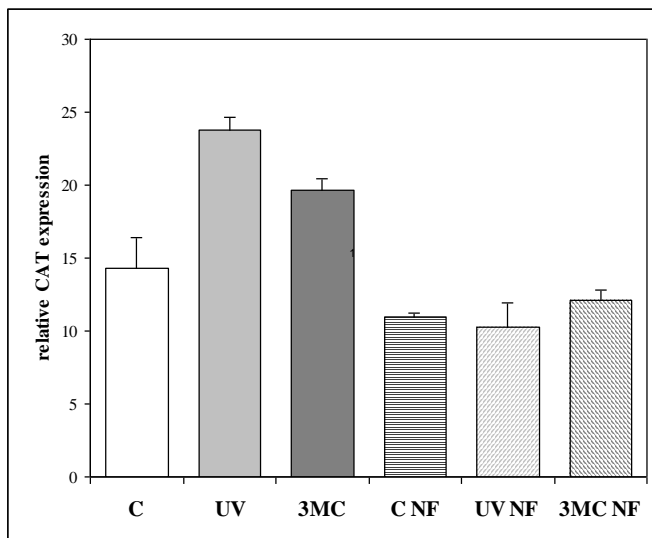
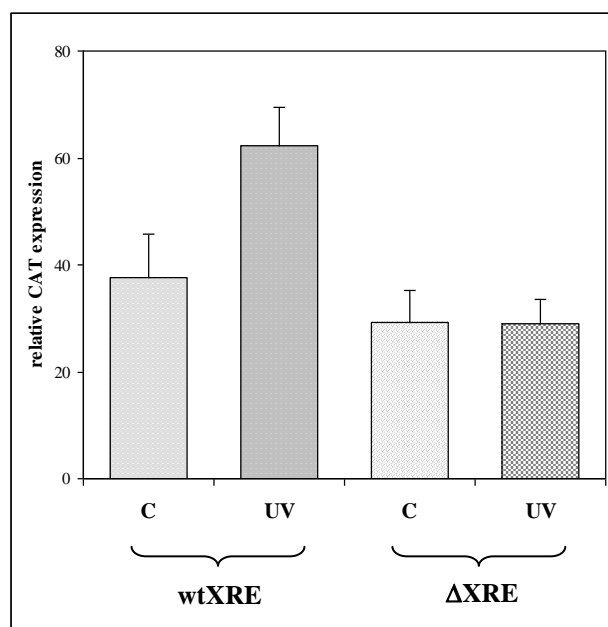
FIG. 1



**FIG. 2**



**A****B****FIG. 4**

**A****B****FIG. 5**

## Legends to Figures

**FIG. 1.** Kinetic of CYP1B1 transcript induction in human keratinocytes and HaCaT cells. (A) Mean ( $\pm$  SD) of kinetics of CYP1B1 transcript induction by UV-B, which were performed on samples from five different donors. Results are expressed as fold increase as compared with each time-control keratinocyte. (B) Kinetics of CYP1B1 transcript induction by UV-B in HaCaT cells.

**FIG. 2.** Inter-individual variability of CYP1B1 mRNA induction by UV-B exposure (20mJ/cm<sup>2</sup>) among 20 individual samples. Each experiment was performed in triplicate.

**FIG. 3.** Role of photoproducts in CYP1B1 transcript induction by UV-B treatment in:

- human keratinocytes (A). C: control keratinocytes. UV: keratinocytes exposed to UV-B. C-NAC: control keratinocytes pretreated with NAC. UV-NAC: keratinocytes pretreated with NAC and exposed to UV-B. Each experiment was performed in triplicate.
- HaCaT cells (B). Control: HaCaT control cells. UV: HaCaT cells exposed to UV-B. Control + NAC: HaCaT control cells pretreated with NAC. UV + NAC: HaCaT cells pretreated with NAC and exposed to UV-B. Control + TRP: HaCaT control cells treated with aqueous tryptophan solution. Control + TRP-UV: HaCaT control cells treated with UV-irradiated tryptophan solution. Each experiment was performed in triplicate.

**FIG. 4.** Effects of  $\alpha$ -amanitin and  $\alpha$ NF on CYP1B1 transcript induction by UV-B exposure on human keratinocyte K18 sample (A) and HaCaT cells (B). Each experiment was

performed in triplicate. C: control cells. UV: cells exposed to UV-B. UV AM: cells exposed to UV-B and treated by  $\alpha$ -amanitin. C NF: control cells treated with  $\alpha$ NF. UV NF: cells exposed to UV-B and treated with  $\alpha$ NF.

**FIG. 5.** Results of CAT assay experiments performed on human keratinocyte K18 sample (A) and HaCaT cells (B). C: control cells. C NF: control cells treated with  $\alpha$ NF. 3MC: cells treated with 3MC. 3MC NF: cells treated with 3MC and  $\alpha$ NF. UV: cells exposed to UV-B. UV NF: cells exposed to UV-B and treated with  $\alpha$ NF. wtXRE: cells transfected with pCAT-(wtXRE promoter).  $\Delta$ XRE: cells transfected with pCAT-( $\Delta$ XRE promoter).