Cytochrome P450 CYP1B1 Interacts with 8-Methoxypsoralen (8-MOP) and Influences Psoralen-Ultraviolet A (PUVA) Sensitivity

Yusuf Y. Deeni1,4, Sally H. Ibbotson2, Julie A. Woods2, C. Roland Wolf1,3, Gillian Smith1*

1 Division of Cancer Research, Medical Research Institute, University of Dundee, Dundee, United Kingdom, 2 Photobiology Unit, University of Dundee, Dundee, United Kingdom, 3 Cancer Research UK Molecular Pharmacology Unit, Ninewells Hospital and Medical School, Dundee, United Kingdom, 4 School of Contemporary Science, University of Abertay Dundee, Dundee, United Kingdom

Abstract

Background: There are unpredictable inter-individual differences in sensitivity to psoralen-UVA (PUVA) photochemotherapy, used to treat skin diseases including psoriasis. Psoralens are metabolised by cytochrome P450 enzymes (P450), and we hypothesised that variability in cutaneous P450 expression may influence PUVA sensitivity. We previously showed that P450 CYP1B1 was abundantly expressed in human skin and regulated by PUVA, and described marked inter-individual differences in cutaneous CYP1B1 expression.

Objectives: We investigated whether CYP1B1 made a significant contribution to 8-methoxypsoralen (8-MOP) metabolism, and whether individuality in CYP1B1 activity influenced PUVA sensitivity.

Methods: We used E. coli membranes co-expressing various P450s and cytochrome P450 reductase (CPR) to study 8-MOP metabolism and cytotoxicity assays in CYP1B1-expressing mammalian cells to assess PUVA sensitivity.

Results: We showed that P450s CYP1A1, CYP1A2, CYP1B1, CYP2A6 and CYP2E1 influence 8-MOP metabolism. As CYP1B1 is the most abundant P450 in human skin, we further demonstrated that: (i) CYP1B1 interacts with 8-MOP (ii) metabolism of the CYP1B1 substrates 7-ethoxyresorufin and 17-β-estradiol showed concentration-dependent inhibition by 8-MOP and (iii) inhibition of 7-ethoxyresorufin metabolism by 8-MOP was influenced by CYP1B1 genotype. The influence of CYP1B1 on PUVA cytotoxicity was further investigated in a Chinese hamster ovary cell line, stably expressing CYP1B1 and CPR, which was more sensitive to PUVA than control cells, suggesting that CYP1B1 metabolises 8-MOP to a more phototoxic metabolite(s).

Conclusion: Our data therefore suggest that CYP1B1 significantly contributes to cutaneous 8-MOP metabolism, and that individuality in CYP1B1 expression may influence PUVA sensitivity.

Introduction

Psoralen-UVA (PUVA) photochemotherapy is widely used to treat psoriasis and other common skin diseases [1,2]. Importantly, PUVA is of superior efficacy to some of the highly costly biologic drugs, with unknown long-term safety, that are increasingly being used to treat psoriasis [3]. There is considerable individuality in PUVA sensitivity, and high cumulative PUVA exposure significantly increases risk of skin cancer [4]. To date, no reliable predictors of PUVA sensitivity have been identified, although there is increasing interest in genetic response predictors [5]. Knowledge of genetic or phenotypic determinants of PUVA sensitivity would facilitate the identification of individuals at increased risk of acute or chronic adverse effects of PUVA treatment.

The most commonly clinically used psoralen is the furanocoumarin, 8-methoxypsoralen (8-MOP), used in systemic and topical PUVA therapy. Previous studies have shown correlations between serum 8-MOP concentrations and PUVA sensitivity, assessed by minimal phototoxic dose (MPD), although there is considerable inter-individual variability in 8-MOP serum levels following oral administration [6–9]. This individuality may reflect inter-individual differences in 8-MOP metabolism, but this also suggests that variation in hepatic P450 gene expression may not be the only contributor and that cutaneous drug metabolising enzymes (DME) may influence PUVA sensitivity. There is considerable evidence that variation in DME, in particular cytochrome P450, activity can be a major determinant of individuality in drug sensitivity [10,11]. In support of this hypothesis, we have shown that PUVA induces the cutaneous expression of several P450s, and reported
marked inter-individual variability in constitutive and inducible P450 expression [12,13].

P450s are a multigene family of inducible Phase I DMEs which, together with their redox partner NADPH cytochrome P450 reductase (CPR), catalyse the oxidative metabolism of many endogenous compounds, drugs and xenobiotics [14–16]. In general, compounds which are P450 inducers are also P450 substrates or inhibitors [17]. P450s have been implicated in psoralen metabolism in insects [18,19] and vertebrates [20], where substrates or inhibitors [17]. P450s have been implicated in general, compounds which are P450 endogenous compounds, drugs and xenobiotics [14–16]. In

%Table 1. Metabolism of 8-MOP by human recombinant P450s and human liver microsomes\(^{a}\).

| P450      | Turnover (min\(^{-1}\)) |
|-----------|-------------------------|
| CYP1A1    | 0.29±0.07               |
| CYP1A2    | 0.63±0.08               |
| CYP1B1    | 0.24±0.05               |
| CYP2A6    | 0.48±0.04               |
| CYP2E1    | 0.08±0.03               |
| CPR       | ND\(^{b}\)              |
| Microsomes\(^{b}\)| 4.73±4.15 |

\(^{a}\)Assays were performed with 5 \(\mu\)M 8-MOP as described in Materials and Methods. Each value represents mean ± SD of three determinations.

\(^{b}\)Mean ± SD of specific activity (n = 3) of microsomes from 3 human livers as pmol/min/mg protein.

\(^{c}\)ND; Not detected.

Co-expression of Human CYP1B1 and CPR in E. coli

Construction of CYP1B1 and CPR expression plasmids has been described previously [25]. The CYP1B1 alleles CYP1B1*1, CYP1B1*3 and CYP1B1*4 were co-expressed with CPR in E. coli JM109 cells and membranes prepared as described previously [26]. P450 content was determined spectrophotometrically [27], and CPR activity estimated by cytochrome c reduction [26]. 8-MOP metabolism was assessed by HPLC analysis, as previously described [26]. Western blot analysis was performed after cells were pre-incubated with 0.05% PBS-EDTA, harvested by trypsinisation (0.25% trypsin-EDTA) and protein content assessed by Bradford assay [29]. Solubilised protein lyases or human liver microsomes were separated by SDS-PAGE, transferred onto Hybond-ECL nitrocellulose membranes (Amersham, UK) and probed with CYP1B1 (BD Gentest, Woburn, MA) and CPR antibodies [30], as previously described [25].

Interaction of 8-MOP with Recombinant P450s

Furanocoumarin metabolism was assessed using a substrate depletion assay, as previously described [31,32]. Assays were performed with 5 \(\mu\)M 8-MOP in 160 \(\mu\)l reaction mixtures containing 50 pmol recombinant P450 membranes or 250 \(\mu\)g human liver microsomes (Tayside Tissue Bank, positive controls) in 50 mM phosphate buffer (PB) pH 7.4. After 3 min 37°C incubation, reactions were initiated by 40 \(\mu\)l 5 mM NADPH containing 30 mM glutathione (GSH) in 50 mM PB (pH 7.4). Reactions were incubated for 10 min in a shaking water bath at 37°C, terminated by the addition of 100 \(\mu\)l ice-cold methanol containing 3% perchloric acid, and placed on ice for 10 min. Zero-time controls were terminated before the addition of P450 membranes; no-NADPH control reactions were initiated with 20 \(\mu\)l 50 mM PB (pH 7.4) containing 30 mM GSH. Samples were vortexed for 1 min, centrifuged at 12 000 rpm for 5 min, supernatants collected and analysed by HPLC analysis [28].

The Influence of 8-MOP on Recombinant Human CYP1B1 Activity

The role of CYP1B1 in 8-MOP metabolism was confirmed using 17β-estradiol 2-hydroxylase, 4-hydroxylase and 7-ethoxyresorufin O-deethylase (EROD) assays. Estradiol hydroxylase assays were performed as previously described [25] using 20 \(\mu\)M 17β-estradiol in the absence (methanol solvent control) or the presence of 8-MOP (1, 10, or 100 \(\mu\)M); EROD activity was determined as previously described [33], but modified in 96-well plate format with quadruplicate replicates, using a Fluoroskan fluorescence reader (Labsystems, Cambridge, UK). Each 200 \(\mu\)l incubation mixture contained PBS, 4 \(\mu\)M 7-ethoxyresorufin, 250 \(\mu\)M NADPH, 5 pmol recombinant CYP1B1, and 8-MOP (0–180 \(\mu\)M, serially diluted). Assays were performed at 37°C, fluorescence readings recorded every 20 s for 3 min at \(\lambda_{\text{ex}} = 530\) nm, \(\lambda_{\text{em}} = 584\) nm and fluorescence production (arbitrary fluorescence units) against time relative to control incubations used to assess 8-MOP inhibition. Calibration curves were constructed with authentic resorufin standards and linear regression analysis used the determination of the amount of resorufin formed in each test sample. Enzyme kinetics for CYP1B1-catalysed EROD activity were determined at substrate concentrations from 0.002 to 2 \(\mu\)M. Apparent \(K_m\) and \(V_{\text{max}}\) values were estimated by non-linear regression analysis (GraFit software program, UK) of the substrate concentration [S]-enzyme activity [V], fitting data to a Michaelis-Menten model. Similarly, the concentration of 8-MOP required to inhibit 50% \((IC_{50})\) of CYP1B1-catalysed EROD at \(K_m\) concentration was calculated. Experiments were performed using
six 8-MOP concentrations 8-MOP (0, 1.17, 2.34, 4.69, 9.38 and 37.50 μM) and four 7-ethoxyresorufin concentrations equivalent to 1/2 K_m, 2 K_m and 5 K_m - mode of inhibition was determined by Lineweaver-Burk plots.

Stable Expression of Human CYP1B1 and CPR in CHO Cells

CHO cell lines stably over-expressing CYP1B1 and CPR were generated as previously described [34]. CHO cells were maintained at 37 °C/5% CO_2 in Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 U/ml each of hypoxanthine and thymine (Invitrogen, Paisley-UK). Similar culture conditions, with the addition of G-418 (400 mg/ml), were used for CHO cells stably expressing CPR (CHO-CPR), CHO cells stably co-expressing CYP1B1 and CPR (CHO-IB1/CPR) were maintained at 37 °C/5% CO_2, but in hypoxanthine and thymine-free DMEM supplemented with 10% dialysed FBS and the appropriate selection (0.3 μM MTX and 400 mg/ml G-418). HaCaT keratinocytes were grown to 80% confluence in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine. Cells were harvested and microsomes prepared as described previously [26]. HaCaT cell microsomes and microsomes from human skin biopsies obtained from peri-lesional skin from psoriasis patients [12,13] were used to investigate functional CYP1B1 expression using EROD assays.

UVA and PUVA Treatment

To investigate CHO cell sensitivity to UVA and PUVA, actively dividing cells (10000 cells/well) were seeded in 96-well plates (Iwaki, Japan) in quadruplicates in 200 μl culture medium and allowed to grow for 18–20 h. Cells were irradiated at room temperature with a UVA source (2×6 ft Cosmolux 15500/100 W, 1.7 mW/cm² (320–400 nm; λmax = 365 nm), Germany) through a 4 mm thick window glass filter at multiple UVA doses (0, 1.5, 4.0 and 7.5 J/cm²), monitoring irradiance with a Waldmann UV meter equipped with UVA detector. Control samples were kept in the dark under the same conditions. To model PUVA treatment, cells were pre-incubated with varying concentrations of 8-MOP (0–5 μM) in PBS at 37°C for 40 min and cells irradiated as described above. Control samples were pre-incubated with solvent and kept in the dark under the same conditions. After each treatment, fresh culture medium was added and cells returned to an

---

**CYP1B1 Allelic Analysis**

| CYP1B1 Allelic Variant | 8-MOP Turnover (min^-1) | IC_50 (μM) | Turnover (ng/ml) |
|------------------------|------------------------|------------|-----------------|
| CYP1B1*1               | 0.27±0.05              | 1.7±0.20   | 369±43          |
| CYP1B1*3               | 0.25±0.03              | 1.5±0.07   | 326±15          |
| CYP1B1*4               | 0.12±0.02              | 0.6±0.10   | 130±22          |

*IC_50 for 8-MOP against CYP1B1-dependent EROD activity determined using *E. coli* membranes co-expressing human individual CYP1B1 alleles and CPR as described in Materials and Methods. Data represents the mean ± SD of triplicate determinations.

---

**CYP1B1 Expression Influences PUVA Sensitivity**

PLOS ONE | www.plosone.org | September 2013 | Volume 8 | Issue 9 | e75494
membranes co-expressing individual P450s present in human skin (CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2E1) and CPR were used to identify P450s involved in 8-MOP metabolism. 8-MOP was metabolized by each of three human liver microsomes, with specific activities of 1.31, 3.53 and 9.55 pmol/min/mg protein, respectively (mean ± SD: 4.73 ± 4.15, Table 1). Each recombinant P450 metabolised 8-MOP in the presence of CPR (Table 1), but not in reactions containing CPR alone or in control experiments omitting CPR or the essential cofactor NADPH (data not shown). 8-MOP metabolism in human liver microsomes and by recombinant CYP1A1, CYP1A2 and CYP1B1 was decreased in the presence of the potent, selective CYP1 P450 family inhibitor α-naphthoflavone (5 μM), where microsomal, CYP1A1, CYP1A2 and CYP1B1-dependent 8-MOP metabolism was reduced to 59, 53, 34 and 62% of control levels, respectively. Of the catalytically active P450s tested, CYP1B1 is most abundantly expressed in human skin [12], and was therefore selected as the focus of additional studies.

### 8-MOP Inhibits CYP1B1 Activity

Using E. coli membranes co-expressing recombinant CYP1B1 and CPR, we showed that 8-MOP inhibited CYP1B1-dependent EROD and 17β-estradiol hydroxylase activities in a concentration-dependent manner (Figures 1A & 1B). The apparent K_m for CYP1B1-dependent EROD activity was 0.33 ± 0.03 μM (n = 5), consistent with previous literature [25]. The addition of 8-MOP decreased CYP1B1-dependent EROD activity in a concentration-dependent manner with an estimated IC_{50} of 2.0 ± 0.1 μM (Figure 1C) and K_i of 3.58 ± 1.00 μM (n = 3). Further, the IC_{50} for 8-MOP metabolism by the low activity CYP1B1*3 allele was significantly reduced relative to the “wild-type” CYP1B1*1, and CYP1B1*3 alleles (Table 2).

To determine the potency and mode of 8-MOP inhibition of CYP1B1, further experiments were performed using six 8-MOP concentrations and four 7-ethoxyresoruﬁn concentrations as described in Materials and Methods. Analysis of enzyme kinetic data by Lineweaver-Burk plots suggested that 8-MOP inhibited CYP1B1 by mixed inhibition (Figure 1D).

### Evidence for the Presence of Catalytically Active CYP1B1 in Human Skin

EROD activity was determined in microsomes isolated from human skin and HaCaT keratinocytes, with turnover rates of 0.10 ± 0.05 and 0.30 ± 0.03 pmol/min/mg protein, respectively. EROD metabolism was significantly reduced in the presence of 5 μM 8-MOP (90% inhibition), and following pre-incubation of HaCaT microsomes with a CYP1B1-specific antibody (60% inhibition) (Figure 2A). In contrast, incubation of microsomes with control CYP3A4 antibodies had no effect on EROD activity. Consistent with our previous data, cutaneous CYP1B1 and CPR expression in non-lesional skin of patients with psoriasis was assessed as described in Materials and Methods. Nuclear CPR localization in human skin was unexpected and may reflect a tissue fixation or processing artefact.

### CYP1B1 and CPR Over-expression Influences PUVA Sensitivity in CHO Cells

We have previously reported that CHO cells have no constitutive P450 and limited CPR expression, but have been engineered to stably over-express CYP1B1 and CPR, individually and in combination [34] (Figure 3A). As described in Materials and Methods, the presence of catalytically active CYP1B1 was confirmed using EROD assays in total protein cell lysates from each cell line. While there was no EROD activity in control CHO
or CHO-CPR expressing cells, CHO-1B1/CPR expressing cells metabolised ethoxy resorufin (EROD activity 1.85 ± 0.87 pmol/min/mg protein; n = 5).

UVA alone at doses up to 7.5 J/cm² and 8-MOP (0–5 μM) alone showed no significant cytotoxicity to CHO cells (Figure 3B & 3C). However, significant cytotoxicity was seen when CHO cells were exposed to 8-MOP and then irradiated with UVA, with dose-dependent effects of both 8-MOP concentration and UVA dose (Figure 3C). Optimal dose-dependency cytotoxicity was seen at a combined UVA dose of 4 J/cm² and an 8-MOP concentration range of 0–5 μM (Figure 3C).

8-MOP alone was not cytotoxic and appeared to promote cell proliferation in each cell line tested, although this was most evident for the catalytically-active CHO-1B1/CPR cell line (Figure 3D). In contrast, PUVA was cytotoxic to all the cell lines tested (Figure 3D).

While the cytotoxic effect of PUVA on the CHO and the CHO-CPR cell lines were similar (p = 0.268, Table 3), the CHO-1B1/CPR cell line was significantly more sensitive to PUVA cytotoxicity than the parental CHO cell line (p = 0.002) or the CHO-1B1/CPR cell line (p<0.001). The addition of catalytically active CYP1B1 to CHO cells resulted in an approximately 2.6-fold increase in PUVA phototoxicity.

Table 3. Modulation of PUVA cytotoxicity in CHO cells by CYP1B1 expression.

| Cell line        | IC50 a (ng/ml) | 8-MOP (μM) | p-Value   |
|------------------|----------------|------------|-----------|
| CHO              | 461±47         | 2.13±0.22  |           |
| CHO-CPR          | 540±48         | 2.50±0.22  | 0.268b    |
| CHO-1B1/CPR      | 241±23         | 1.11±0.11  | 0.002b    |

*aIC50 for 8-MOP in the presence of UVA (4 J/cm²) determined as described in Materials and Methods. Data represents the mean ± SE of 5 experiments.

**p-value as compared with control CHO cell line.

p-value as compared with CHO-CPR cell line.

Table 3. Modulation of PUVA cytotoxicity in CHO cells by CYP1B1 expression.

PLOS ONE | www.plosone.org 5 September 2013 | Volume 8 | Issue 9 | e75494

Discussion

Psoralens, particularly systemic and topical 8-MOP, are widely used in dermatology in PUVA photochemotherapy. However, the enzymes involved in 8-MOP metabolism have not been fully characterised, and it is not known whether individuality in 8-MOP metabolism may influence PUVA sensitivity. Previous studies have described O-demethylation, hydroxylation and epoxidation as important mechanisms of 8-MOP metabolism [6,36]. This, together with the observation that phenobarbital and α-naphthoflavone induce 8-MOP metabolism in vivo and in vitro [36], suggest a role for P450s in 8-MOP metabolism, and an inhibitory effect of furanocoumarins on human CYP1A2 has recently been confirmed [21]. However, with the exception of the predominantly hepatic CYP2A6 [37,38] and respiratory tract-specific CYP2A13 [23,39], cutaneous P450s active in 8-MOP metabolism have not been characterised.

We have previously shown that the cutaneous expression of several P450s, including CYP1B1, CYP2S1 and CYP2E1, is regulated by PUVA in vivo, and have shown marked individuality in gene expression [12]. CYP1B1 is the most abundantly expressed P450 in human skin, and furanocoumarins have been shown to inhibit CYP1B1 activity [22]. 8-MOP is highly structurally similar to the CYP2A6 substrate coumarin, another competitive CYP1B1 inhibitor [40], suggesting that cutaneous CYP1B1 expression may...
influence PUVA sensitivity. We found that 8-MOP inhibits CYP1B1-dependent EROD and 17-β-estradiol hydroxylase activities in a concentration-dependent manner, with estimated IC_{50} values for EROD similar to those reported for another furanocoumarin, bergamottin [41]. Moreover, the inhibition of CYP1B1-dependent EROD metabolism by 8-MOP was demonstrated in both human skin microsomes and in a human keratinocyte cell line. These data suggest that 8-MOP may be a CYP1B1 substrate and demonstrate that 8-MOP and/or its metabolite(s) are potent CYP1B1 inhibitors.

The inhibition of CYP1B1 by 8-MOP and/or metabolites could occur by direct selective inhibition and/or mechanism-based inactivation as reported with other P450s [21]. Previously, 8-MOP and other furanocoumarin derivatives have been shown to be potent mechanism-based P450 inhibitors [38,42], with the generation of a reactive intermediate by initial oxidation of the furan ring to form furanepoxide intermediates that readily react with target residue(s) in the active site of the P450 [24]. Currently, it is not known whether 8-MOP inhibits the catalytic activity of CYP1B1 in vivo, however clinical data suggest that this is likely [37,43].

We were therefore interested to find that, using a modified CHO cell line stably over-expressing recombinant human CYP1B1 together with its redox partner CPR, CHO-1B1/CPR cells were significantly more susceptible to PUVA cytotoxicity than control cell lines. These data strongly suggest that CYP1B1 may metabolise 8-MOP to product(s) which are more phototoxic than the parent compound. In support of this hypothesis, we have recently shown that CYP1B1 is induced by UVB, but is not significantly induced by UVA in HaCaT keratinocytes [29], consistent with increased PUVA sensitisation arising from altered 8-MOP metabolism. Evidence from several cell line models additionally suggests that PUVA is a source of reactive oxygen species leading to oxidative stress [44], genotoxic effects and the formation of DNA photodadducts [45]. It is therefore conceivable that the increased PUVA cytotoxicity in the CHO-1B1/CPR cell line may reflect increased genotoxicity and DNA photodadduct formation and this requires further study.

CYP1B1 is polymorphic, with a population distribution of alleles with altered catalytic activity [46]. In particular, the CYP1B1*3 allele (Val143Leu) allele has been shown to significantly influence the conversion of estradiol to 4-hydroxyestradiol [25] and the CYP1B1*4 allele (Asn432Ser) to have reduced protein expression and catalytic activity, as a consequence of enhanced polyubiquitin-mediated degradation [47]. Our finding that 8-MOP metabolism is also impaired in cells expressing the CYP1B1*4 allele is therefore intriguing, and suggests that both genetically determined and transcriptionally regulated inter-individual differences in CYP1B1 activity may influence PUVA sensitivity. As PUVA is photocarcinogenic, with high cumulative PUVA exposure incurring a significantly increased risk of skin cancer [4], it will be of particular interest in future clinical studies to investigate whether CYP1B1 genotype influences PUVA-associated skin cancer risk, and whether CYP1B1 genotype or phenotype influences PUVA sensitivity.

References

1. Norris PG, Hask JLM, Baker C, Bihland D, Difley BL, et al. (1994) British Photodermatology Group Guidelines for PUVA. British Journal of Dermatology 130: 246–255.

2. Patel RV, Clark LN, Lebwohl M, Weinberg JM (2009) Treatments for psoriasis and the risk of malignancy. Journal of the American Academy of Dermatology 60: 1001–1017.

3. Inzinger M, Heschl B, Weger W, Hofer A, Legat FJ, et al. (2011) Efficacy of psoralen plus ultraviolet A therapy vs. biologics in moderate to severe chronic plaque psoriasis: retrospective data analysis of a patient registry. British Journal of Dermatology 165: 640–645.

4. Stern RS, Laird N (1994) The Carcinogenic Risk of Treatments for Severe Psoriasis. Cancer 73: 2759–2764.

5. Bobson SH, Dowse RS, Dinkwa-Kostova AT, Weidlich S, Farr PM, et al. (2012) Glutathione S-transferase genotype is associated with sensitivity to psoralen-ultraviolet A phototherapy. British Journal of Dermatology 166: 300–308.

6. de Wolff FA, Thomas TV (1986) Clinical pharmacokinetics of methoxsalen and other psoralens. Clin Pharmacokinet 11: 62–73.

7. Goldstein DP, Carter DM, Ljunggren B, Burdhohler J (1982) Minimal phototoxic doses and 8-MOP plasma levels in PUVA patients. Journal of Investigative Dermatology 78: 429–433.

8. Marrakchi S, Decloquement L, Pollet P, Briand G, Loesche C, et al. (1991) Variation in 8-methoxypsoralen profiles during long-term psoralen plus ultraviolet-A therapy and correlations between serum 8-methoxypsoralen levels and chromatomic parameters. Photodermatology Photoinmunology & Photomedicine 8: 206–211.

9. McLeod J, Fisher C, Farr PM, Difley BL, Cox NH (1991) The relationship between plasma psoralen concentration and psoralen UVA erythema. British Journal of Dermatology 124: 585–590.

10. Chen Q, Zhang T, Wang JF, Wei DQ (2011) Advances in human cytochrome P450 family and personalized medicine. Current drug metabolism 12: 436–444.

11. Wolf CR, Smith G (1999) Pharmacogenetics. British Medical Bulletin 55: 366–388.

12. Smith G, Dawe RS, Clark C, Evans AT, Comrie MM, et al. (2003) Quantitative real-time reverse transcription-polymerase chain reaction analysis of drug metabolizing and cytoprotective genes in psoriasis and regulation by ultraviolet radiation. Journal of Investigative Dermatology 121: 390–398.

13. Smith G, Wolf CR, Deenl YY, Dawe RS, Evans AT, et al. (2003) Cutaneous expression of cytochrome P450CYP2S1: individuality in regulation by therapeutic agents for psoriasis and other skin diseases. Lancet 363: 1336–1343.

14. Ahmad N, Agarwal R, Mukhtar H (2004) Cytochrome P450 and drug development for skin diseases. Skin Pharmacology 9: 231–241.

15. Ahmad N, Mukhtar H (2004) Cytochrome P450: a target for drug development for skin diseases. J Invest Dermatol 123: 417–425.

16. Guengerich FP (2003) Cytochromes P450, drugs, and diseases. Mol Interv 3: 380–388.

17. Nebert DW (2000) Drug-metabolizing enzymes, polymorphisms and interindividual response to environmental toxicants. Clin Chem Lab Med 38: 857–861.

18. Li W, Schuler MA, Berenbaum MR (2003) Diversification of furanocoumarin-metabolizing cytochrome P450 monooxygenases in two papilionids: Specificity and substrate encounter rate. Proc Nat Acad Sci U S A 100 Suppl 2: 14593–14598.

19. Wei Z, Pan L, Berenbaum MR, Schuler MA (2003) Metabolism of linear and angular furanocoumarins by Papilio polyxenes CYP6B1 co-expressed with NADPH cytochrome P450 reductase. Insect Biochem Mol Biol 33: 937–947.

20. Guo LQ, Yanamato Y (2004) Inhibition of cytochrome P450 by furanocoumarins in grapefruit juice and herbal medicines. Acta Pharmacol Sin 25: 129–136.

21. Kang A-Y, Young L, Dingfelder C, Peterson S (2011) Effects of Furanocoumarins from Apiaceous Vegetables on the Catalytic Activity of Recombinant Human Cytochrome P450 1A2. The Protein Journal 30: 447–456.

22. Giremava B, Poulose SM, Jayaprakasha GK, Bhat NG, Paill BS (2006) Furocoumarins from grapefruit juice and their effect on human CYP 3A4 and CYP 1B1 isoenzymes. Bioorganic & Medicinal Chemistry 14: 2606–2612.

23. Guo T, Morishita H, Fu XS, Bergasa T, Matushara T, et al. (2010) The Effects of Single Nucleotide Polymorphisms in CYP2A13 on Metabolism of 5-Methoxypsoralen. Drug Metabolism and Disposition 38: 2110–2116.

24. Ueng Y-F, Chen C-C, Chung Y-T, Lin E-Y, Chang Y-P, et al. (2011) Mechanism-based inhibition of cytochrome P450 (CYP2A6) by chalpepen in recombinant systems, in human liver microsomes and in mice in vivo. British Journal of Pharmacology 163: 1250–1262.

25. Li DN, Seidel A, Pritchard MF, Wolf CR, Friedberg T (2000) Polymorphisms in CYP 1B1 isoenzymes. Bioorganic & Medicinal Chemistry 14: 2606–2612.

26. Gu D, Lin SD, Seidel A, Pritchard MF, Wolf CR, Friedberg T (2000) Polymorphisms in CYP1B1. J Invest Dermatol 121: 390–398.

27. Li D, Seidel A, Pritchard MF, Wolf CR, Friedberg T (2000) Polymorphisms in CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. Pharmacogenetics 10: 343–353.
26. Deeni YY, Paine MJI, Aytoon AD, Clarke SE, Clenbery R, et al. (2001) Expression, Purification, and Biochemical Characterization of A Human Cytochrome P450 CYP2D6-NADPH Cytochrome P450 Reductase Fusion Protein. Archives of Biochemistry and Biophysics 396: 16–24.

27. Omura T, Sato R (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J Biol Chem 239: 2370–2378.

28. Gasparro FP, Battista J, Song J, Edelson RI (1983) Rapid and sensitive analysis of 8-methoxypsoralen in plasma. Journal of Investigative Dermatology 90: 234–236.

29. McNeely AD, Woods JA, Ibbotson SH, Wolf CR, Smith G (2011) Characterisation of a Human Keratinocyte HaCaT Cell Line Model to Study the Regulation of Cytochrome P450 CYP2S1. Drug Metabolism and Disposition 40: 283–289.

30. Smith GC, Tew DG, Wolf CR (1994) Dissection of NADPH-cytochrome P450 oxidoreductase into distinct functional domains. Proceedings of the National Academy of Sciences 91: 8710–8714.

31. Li W, Zangerl AR, Schuler MA, Berenbaum MR (2004) Characterization and evolution of furanocoumarin-inducible cytochrome P450s in the parsnip webworm, Depressaria pastinacella. Insect Molecular Biology 13: 603–613.

32. Obach RS, Reed-Hagen AE (2002) Measurement of Michaelis constants for cytochrome P450-mediated biotransformation reactions using a substrate depletion approach. Drug Metab Dispos 30: 831–837.

33. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T, et al. (1985) Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. Biochem Pharmacol 34: 3337–3345.

34. Ding S, Yao D, Deeni YY, Burchell B, Wolf CR, et al. (2001) Human NADPH- P450 oxidoreductase modulates the level of cytochrome P450 CYP2D6 holoprotein via haem oxygenase-dependent and -independent pathways. Biochem J 356: 613–619.

35. Triglia D, Braa SS, Yonan C, Naughton GK (1991) In vitro toxicity of various classes of test agents using the neutral red assay on a human 3-dimensional physiological skin model. In Vitro Cellular & Developmental Biology 27: 239–244.

36. Mays DC, Hecht SG, Unger SE, Pacula CM, Clainie JM, et al. (1987) Disposition of 8-methoxypsoralen in the rat. Induction of metabolism in vivo and in vitro and identification of urinary metabolites by thermospray mass spectrometry. Drug Metab Dispos 15: 318–328.

37. Kharsa J, Hanks J, Taraday JK (2000) Single-dose methoxsalen effects on human cytochrome P-450 2A6 activity. Drug Metab Dispos 28: 20–33.

38. Koenigs LL, Trager WF (1998) Mechanism-based inactivation of P450 2A6 by furanocoumarins. Biochemistry 37: 10047–10061.

39. von Weymarn LR, Chun JA, Hollemborg PF (2006) Effects of benzy1 and phenethyl isothiocyanate on P450s 2A6 and 2A13: potential for chemoprevention in smokers. Carcinogenesis 27: 782–790.

40. Mammen JS, Kleinle HR, DiGiovanni J, Sutter TR, Strickland PT (2005) Coumarins are competitive inhibitors of cytochrome P4501B1, with equal potency for allelic variants. Pharmacogenetics and Genomics 15: 183–188.

41. Kleiner HE, Valimiri SV, Reed MJ, Uberecken A, DiGiovanni J (2002) Role of cytochrome P450 1A1 and 1B1 in the metabolic activation of 7,12-dimethylbenz[a]anthraene and the effects of naturally occurring furanocoumarins on skin tumor initiation. Chem Res Toxicol 15: 226–235.

42. Koenigs LL, Trager WF (1998) Mechanism-based inactivation of cytochrome P450 2B1 by 8-methoxypsoralen and several other furanocoumarins. Biochemistry 37: 13184–13193.

43. Tantcheva-Poor I, Servera-Llaneras M, Scharffetter-Kochanek K, Fuhr U (2001) Liver cytochrome P450 CYP4A2 is markedly inhibited by systemic but not by bath PUVA in dermatological patients. Br J Dermatol 144: 1127–1132.

44. Mukhtar H, Elmers CA (1996) Photocarcinogenesis: Mechanisms, models and human health implications. Introduction. Photochemistry and Photobiology 63: 355–357.

45. Papadopoulou D, Averbeck D (1983) Genotoxic effects and DNA photoadducts induced in Chinese hamster V79 cells by 5-methoxypsoralen and 8-methoxypsoralen. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 151: 281–291.

46. Shihada T, Watanabe J, Kasajiri K, Sutter TR, Guengerich FP, et al. (1999) Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 20: 1607–1614.

47. Bandiera S, Weidlich S, Harth V, Broede P, Friedberg T (2003) Proteasomal degradation of human CYP1B1: Effect of the Aur53Ser polymorphism on the post-translational regulation of CYP1B1 expression. Molecular Pharmacology 67: 433–443.