

# Cellular and genomic toxicity produced by UV light in Chinese hamster ovary cells

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**Abstract:** UVB and UVC toxicity was detected in Chinese Hamster Ovary (CHO) cell lines AA8, UV5 and XEM2 (a V79-derived cell line expressing rat P 450 1A1). Unlike FICZ-HPLC assay that showed induction of CYP1A1 enzyme activity after 20 minutes and 2 hour UVC exposure, the EROD assay showed no difference in cytochrome P450 1A1 (CYP1A1) activity after exposure to different doses of UVB and UVC light. Different cytotoxic and mutagenic effect of photo lesions induced by UVC and UVB light was investigated with the DRAG and HPRT assays, comparing the wild type cell line AA8 and the Nucleotide Excision Repair (NER) deficient cell line UV5. DRAG assay showed a significant difference in UV induced cytotoxicity between UVC and UVB reflecting the larger energy and toxic effect of UVC along with significant difference in UV induced toxicity between AA8 and UV5 cell lines. This was further validated through the HPRT assay, which also showed a significant difference in UVC (5 J/m<sup>2</sup>) induced mutagenic effect between these cell lines. In addition, HPRT assay showed the mutagenic effect of photosensitizer, acetophenone. These results show that UVB and UVC generate serious damage through photo products on DNA, and might induce the metabolic activity of CYP1A1.

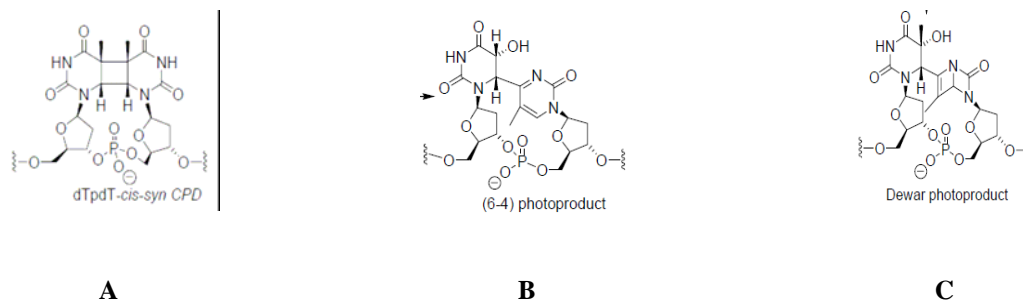
**Keywords:** UV light; cytotoxicity; P450 1A1; AA8; DRAG; HPRT.

## INTRODUCTION

All living organisms are exposed to sun light and the ultra violet (UV) light present in the sun is known to be mutagenic and carcinogenic (Urbach, 1997). Three categories of UV with different wavelength regions: UVA (320-400nm), UVB (290-320nm) and UVC (100-290nm) causes distinct genomic and cellular damage. The depth of penetration into the skin is associated with the wavelength, the longer the wavelength, the deeper the penetration (Ibrahim and Brown, 2008; Latonen and Laiho, 2005). UVA has a relatively longer wavelength and cannot be absorbed by the ozone layer. About 90-95% of solar UV radiation is UVA. UVA penetrates deeper into the epidermis and dermis of the skin at a depth of approximately 1000  $\mu$ m and is not strongly absorbed by DNA. However, it can generate reactive oxygen species (ROS) which causes oxidative DNA damage and leads to skin aging and cancers such as melanoma. Most of UVB is absorbed by the ozone layer; still about 5% of solar radiation to the earth is from UVB. UVB radiation can penetrate the skin to a depth of 160-180  $\mu$ m and can be absorbed by proteins and nucleic acids. Many severe damages on skin are related with UVB, such as sunburns, skin aging and cancers. Almost all UVC is absorbed by ozone layer before it reaches the surface of the earth. However, because of more and more serious pollution, the ozone layer becomes thin and the ozone holes are formed that lead to increased shorter wavelength of UV light onto

the earth, including UVB and UVC (Mckenzie, 1995). UVC generates high energy that is more mutagenic and toxic to cells. UVC can penetrate the skin to a depth of 60-80  $\mu$ m and can also be absorbed by DNA where it causes more serious damage (Petitfrere *et al.*, 1996). Therefore, UVC is used for air, surface and water disinfection. When cells are exposed to UV light, the energy of photons is absorbed by biomolecules such as melanin, DNA, amino acids, carotene and urocanic acids (Anderson and Parrish, 1981). These biomolecules consume the energy and may change their structures. In the skin, DNA is the most critical molecule responding to UV radiation. Generally, intra-strand dimers are formed by the reaction between nucleobases and their immediate neighboring counterparts. The most common nucleobase is pyrimidine which usually forms CC-dimers, CT/TC-dimers and TT-dimers (Taylor, 1990). The CC-dimer is not chemically stable, and tautomerizes or deaminated to U. A is directed by U of a dimer that results C to T mutation (Nan Jiang, 1993). Since DNA absorbs UV light photon energy, cells are more vulnerable to UVC due to high energy photon. The common photoproducts induced by UVC are cis-syn cyclobutane pyrimidine dimers (CPDs) (fig. 1a) and pyrimidine (6-4) pyrimidone photoproducts [(6-4)PP] (fig. 1b). Besides these two products, UVB also induces Dewar isomers (fig. 1c) which are produced from the (6-4) PP by wavelengths longer than 290 nm (Clingen *et al.*, 1995). It has been reported that UV-induced DNA adducts, CPD and 6-4PP, can affect cell apoptosis and cell cycle arrest (Hsin-Lung

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**Fig. 1:** Structure of dTpdT photoproducts (Clingenet *et al.*, 1995)

*et al.*, 2005). Incorrect repair of these products can cause gene mutation and trigger cytotoxicity by affecting gene transcription and replication. In addition, UV radiation also induces non-dimer photoproducts such as cytosine photohydrates, purine photoproducts and single-strand breaks in DNA (Ananthaswamy and Pierceall, 1990).

About 90 percent of non-melanoma skin cancers are associated with exposure to UV radiation from the sun (Abdulla *et al.*, 2005). In this study, we performed four assays to analyze UVB and UVC damage to Chinese Hamster Ovary (CHO) Cells, including EROD test, and utilizing HPLC on metabolic activity of CYP1A, HPRT-gene mutagenic potency experiment and DRAG analysis to measure basal cytotoxicity depending on the gene expression pattern of the cells.

## MATERIAL AND METHODS

### Cell lines and cell culture

The cell lines AA8 and UV5 were used for the cytotoxicity test and mutagenic potency experiment. The AA8 cells are wild type CHO cells while UV5 cells carry a deficiency in NER due to a mutation in ERCC2/XPD. XEM2 cells were used to analyze the effects on metabolic activity and measure cellular metabolites. XEM2 cells express CYP1A1 since they are stably transfected with rat CYP1A1 gene inserted in the genome. Cells were cultivated in DMEM (10%PBS, 1%PEST, 1% HEPES) and were incubated at 37°C in 5% CO<sub>2</sub>.

### Determination of cytochrome P450 1A1 activity by using the EROD assay

In EROD assay, the enzyme acts on substrate (etoxyresorufin) and will upon metabolism yield a product (resorufin) with a known excitation and emission of light (535nm and 590nm). EROD and MROD are markers of cytochrome P4501A activities (Zamaratskaia and Zlabek, 2009) and this method was used to measure the P450 1A1 activity by detecting the product named resorufin, which is produced from the substrate etoxyresorufin. Resorufin concentration was measured in the Polar Star Omega spectrophotometer, with excitation and emission pattern (535resp. 590nm). 10000 XEM2 cells per well were seeded in two strips of 8 wells. After

changing the medium with HBSS++ without phenol red, the cells were exposed in 100 ul HBSS to UVB (0, 200,400, 800J/m<sup>2</sup>) and UVC (0, 2.5, 5, 10J/m<sup>2</sup>), 2 wells per dose. The substrate Etoxyresorufin 100 ul was added in each well and then strips were run for 3 hours in the spectrophotometer. Finally, the wells were washed by HBSS++ and exposed to Rezasurin (10µg/ml) for 1 hour following by measurement the fluorescence at 535/590nm, to determine cell concentration in each well, individually.

### Determination of cytotoxic potency using the DRAG assay

The DRAG assay is used to detect genotoxic damage (Helleday *et al.*, 2001) and is a useful tool for the detection of repairable adducts which is based on the inhibition of the growth of DNA repair-deficient CHO cells. 3,000 AA8 and UV5 cells in 200 ul DMEM per well were seeded in a 96 well plate. Four rows were seeded with AA8 cells and the next four rows were seeded with UV5 cells. Column 12 remained empty as a control to measure the background fluorescence of the plastic. After 24h incubation, cells were exposed to a series of doses of UV light (UVB:0, 50, 100, 200, 400, 600, 800, 1000, 2000, 4000, 8000J/m<sup>2</sup>; UVC: 0, 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 30J/m<sup>2</sup>). Column 1 was not irradiated and was used as control for normal cell growth. After 72h of incubation in DMEM, the cells were exposed to Rezasurin for 60 min and the fluorescence, which is proportional to the amount of cells in the well, was measured at 535/590 nm.

### HPRT gene mutation assay

In order to study induction of mutations from different types of UV light, the *hprt*-gene was used for selection of mutant cell colonies. The *hprt* protein is a non-essential transferase involved in the recycling process of DNA bases, coupling a phosphate group onto guanine. Selection of mutant colonies is made by adding the toxic 6-thio-guanine (6-TG) to the media. 6-TG is mistakenly incorporated onto the DNA due to the *hprt* protein, leading to the survival of colonies mutant in *hprt*-gene only (Vrieling *et al.*, 1989).

100 000 AA8 or UV5 cells per dish were seeded in 5 small dishes. After 24 hours incubation in 5 ml DMEM,

the cells were exposed to UVC, UVB, UVC +0.2% Acetophenone, UVB +0.2% Acetophenone, with one untreated control. The dose of UVB is 800J/m<sup>2</sup> and that of UVC is 10J/m<sup>2</sup>. After 24 hours recovery incubation, for clonogenic survival dishes, 200 cells were reseeded in each dish and two dishes for each treatment group. For expression growth, 200 000 AA8 and 300 000 UV5 cells in each flask were seeded in two flasks in 20 ml DMEM for each treatment group. The clonogenic survival dishes were incubated for 5 days, then fixed and stained with methylene blue in methanol and colonies were counted. The expression growth flasks were incubated for 4 days and reseeded onto new duplicate flasks (duplicates were mixed before reseeding) and incubated for 3 days. The cells were then rinsed with HBSS<sup>+</sup> and reseeded (duplicates were mixed before reseeding) onto triplicate petri dishes for selection in DMEM with 5μl/ml 6-TG and onto duplicate petri dishes for clonogenic survival in DMEM (without 6-TG). The petri dishes were incubated for 6 days, then fixed and stained with methylene blue in methanol and colonies were counted.

#### Evaluation of Cytochrome P450 1A1 inhibitor by HPLC

HPLC is used to determine cytochrome P450 1A activities (Pegolo *et al.*, 2010) and in this study it was used to investigate whether the metabolism of FICZ in XEM2 cells could be affected after exposure of UV light. XEM2 cells were irradiated to UVB at a dose of 20mJ/cm<sup>2</sup> or UVC at a dose of 2.5mJ/cm<sup>2</sup> in 2ml PBS. After UV treatment, PBS was exchanged with 5ml medium containing 10 nM FICZ. The control series was treated in the same way but not irradiated to UV. After 20 minutes and 2 hours incubation at 37°C in the dark respectively, the cells were washed with PBS thereafter scraped in 1000μl dH<sub>2</sub>O followed by sonication. Samples were stored at -20°C until further analysis by HPLC. A RAM-column and a reverse phase C18 column were used for the HPLC analysis.

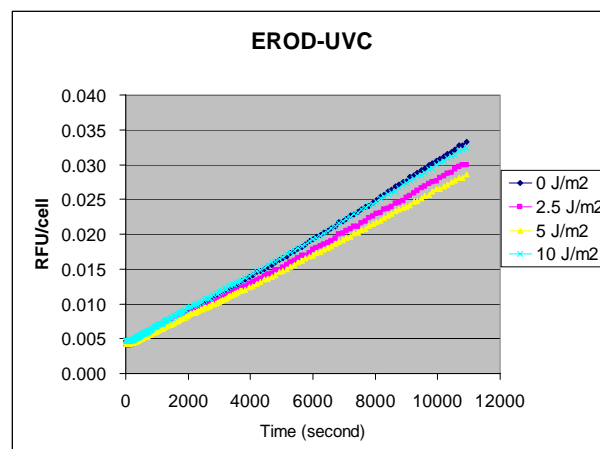
#### STATISTICAL ANALYSES

All values were expressed as means ± SD. Statistical analyses were performed using SAS statistical software, version 8.0 (SAS Institute, Cary, NC, USA) for Windows<sup>®</sup>. A *p*-value ≤0.05 was considered to be statistically significant.

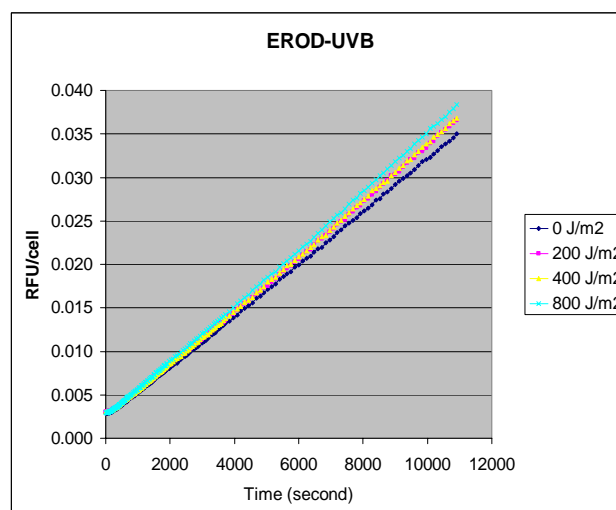
#### RESULTS

##### Cytochrome P450 1A1 activity is not affected by UVB and UVC in XEM2 cells

The EROD assay showed kinetic reaction of the enzyme P450 1A1 metabolism of etoxyresorufin. Relative fluorescence units (RFU)/cell for resorufin were linear with reaction time (figs. 2 a,b). But no significant difference was observed between the control and treatment group (*p*>0.05). At these doses, UVC and UVB irradiation did not influence P450 1A1 activity.

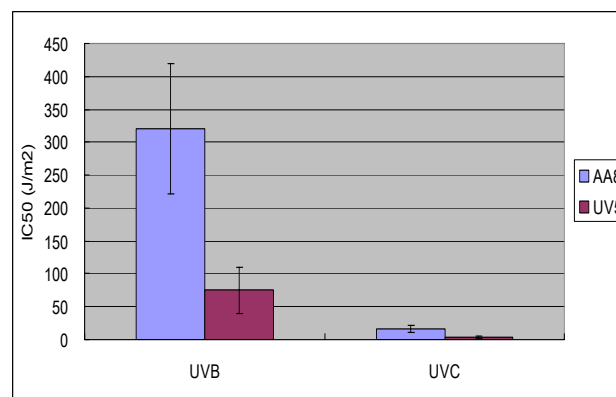


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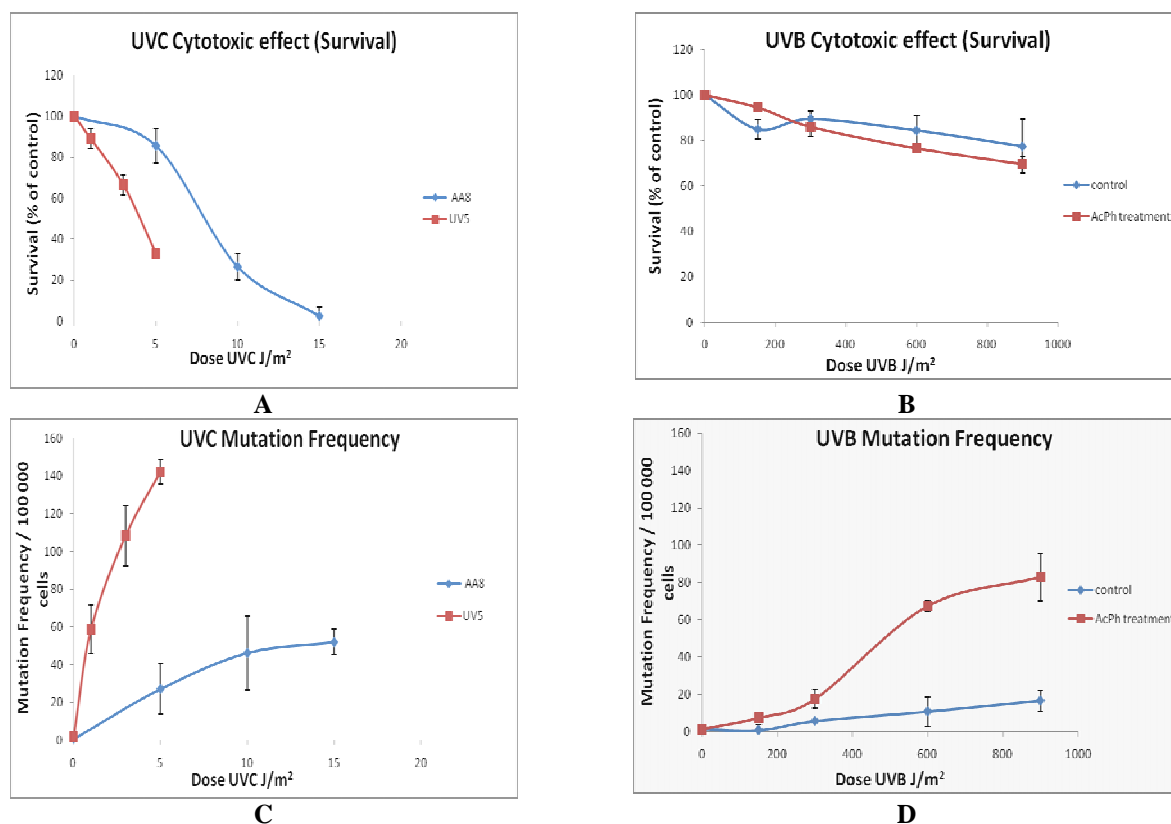


B

**Fig. 2a & b:** EROD results of XEM2 cells exposed to UVC from 0-10J/m<sup>2</sup> and UVB from 0-800J/m<sup>2</sup>.



**Fig. 3:** IC<sub>50</sub> values for UVB and UVC in NER<sup>+</sup> and NER<sup>-</sup> cells. IC<sub>50</sub> for AA8 cells that were exposed to UVB is 321, however the IC<sub>50</sub> for NER deficient cells exposed to UVB is significantly lower that is 75 (*p*=0.003). When AA8 cells were exposed to UVC the IC<sub>50</sub> is 16, which is significantly higher than the IC<sub>50</sub> of NER deficient cells that is 3.7 (*p*=0.001).



**Fig. 4:** a) Cytotoxic effect with increasing UVC dose, UV5 cell line being 14.1% more sensitive than the AA8 cell line, difference being significant. b) Cytotoxic effect with increasing UVB dose, cell line treated with the photosensitizer Acetophenone (0.2%) showing no significant difference in sensitivity compared to the cell line without Acetophenone (0.2%) treatment. c) Mutagenic effect with increasing UVC dose, mutations in response to UVC dose being higher in the UV5 cell line than in the AA8 cell line. d) Mutagenic effect with increasing UVB dose, mutations in response to UVB dose being higher in treated cell line than in untreated cell line.

**Different cytotoxicity produced by UVB and UVC in AA8 and UV5 cells**

The DRAG assay was performed to investigate the amount of cell damage repaired by NER. At different doses of UVB and UVC irradiation, AA8 cells and UV5 cells growth were influenced differently because of their distinct DNA damage repair ability (UV5 cells were NER deficient). The dose values giving cell growth reduction of 50% (IC<sub>50</sub>) were calculated that IC<sub>50</sub> of AA8 was nearly 10 times of UV5 for UVB and 4 times for UVC. It proved that NER is important for repair of DNA damage caused by both UVB and UVC light (fig. 3).

**Determination of HPRT-gene mutation caused by UVB and UVC**

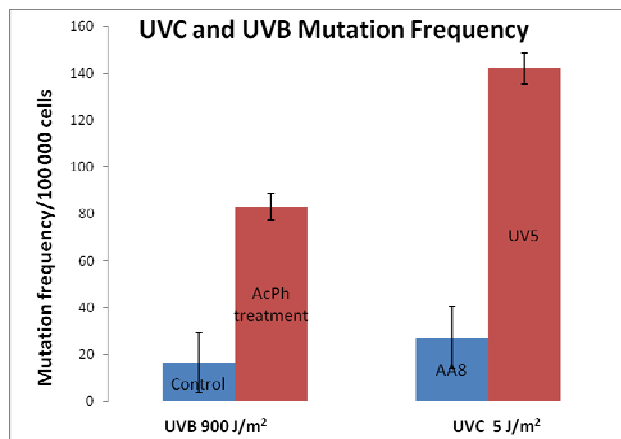
This assay included two parts: one was the detection of cytotoxic effects (figs. 4a, b) and other was the induction of mutations (figs. 4c, d), both from different spectra of UVB and UVC irradiation. Exposed to the same dose of UVC, the survival rate of UV5 was much lower than AA8 (fig. 4a, IC<sub>20</sub> UV5 cell line 0.98 J/m<sup>2</sup>, IC<sub>20</sub> AA8 cell line 6.9 J/m<sup>2</sup>, p=0.02). However, the photosensitizer (0.2% AcPh) did not influence the survival rate of AA8 cells

which were exposed to UVB (fig. 4b, IC<sub>20</sub> cell line with AcPh 764 J/m<sup>2</sup>, IC<sub>20</sub> AA8 cell line 540 J/m<sup>2</sup>, p=0.45). Much higher mutation frequency was found in UV5 (137.4) compared to AA8 (36.5) when they were exposed to the same dose (5J/m<sup>2</sup>) of UVC (fig. 4c). The photosensitizer (0.2% AcPh) increased the mutation frequency in AA8 cells when they were exposed to different doses of UVB (figs. 4d, 6). Compared with UVB, UVC caused more mutations in the same cell line (AA8) in the given spectra of doses (fig. 5).

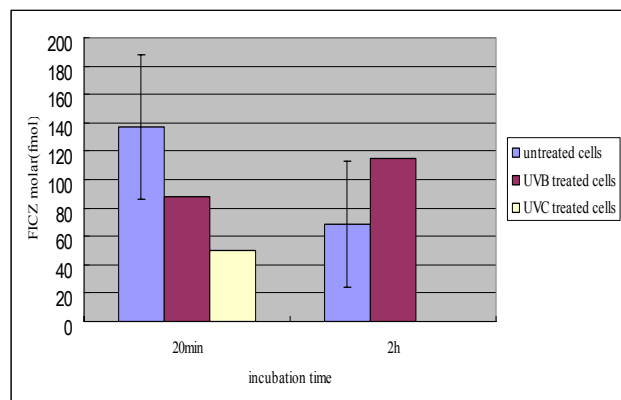
**Determination of cytochrome P450 1A1 activity by detecting FICZ metabolism**

This assay detected the remaining amount of FICZ in non-treated and UV-treated XEM2 cells after incubating in medium with FICZ for 20 minutes and 2 hours. The retention time of FICZ peak in the HPLC column was 23.7 minutes. The area of the peak was calculated automatically as mFU.s and the value of standard FICZ sample was 6.64 mFU.s/fmol. According to the volume injected in the column, the molar values of FICZ left in the cells were calculated (fig. 6). Only the value in UVB treated cells for 2 hour incubation was higher than non-

treated cells, others were lower instead. The results showed P450 1A1 activity could be induced by UVC. But for UVB, the FICZ amount in treated cells after 2 hours was higher than after 20 minutes, so it could not be drawn any conclusion for UVB effect on P450 1A1 activity in XEM2 cells.



**Fig. 5:** Difference between UVC and UVB, UVC being much more mutagenic. Significant difference between cell lines with and without acetophenone (0.2%) treatment ( $p=0,021$ ), UVB treated. Also significant difference between UV5 and AA8 cell lines ( $p=0.00813$ ), UVC treated.



**Fig. 6:** The remaining amount of FICZ in untreated and UV-treated XEM2 cells after incubating with FICZ in dark for 20 minutes and 2 hours.

## DISCUSSION

Induction of cytochrome P450 1A1 (CYP1A1) activity by UV light has been reported previously (Nouspikel, 2009; Reardon and Sancar, 2005). At the gene expression level, UVB induced increase in CYP1A1 mRNA in the mouse wild type Hepa-1 cells at the doses 200 and 400J/m<sup>2</sup>. It has also been demonstrated that UV-induced CYP1A1 gene expression is aryl hydrocarbon receptor (AhR) dependent (Nouspikel, 2009). Tryptophan is one of the most strongly near-UV absorbing amino acid. Upon UV irradiation of tryptophan, a variety of photoproducts are formed. FICZ is one of the photoproducts which display

an extremely strong affinity toward the AhR (Tornaletti, 2009; Rannug *et al.*, 1987). AhR plays a central role in the induction of some AhR-regulated genes including drug-metabolizing enzymes such as the cytochrome P450 1A1. The EROD assay for UV light showed no effect in treated cells compared to untreated cells. However, in FICZ-HPLC assay, the remaining of FICZ in the UVC treated XEM2 cells was much lower than the untreated cells after 20 minutes, and 2 hour incubation. These results indicated that CYP1A1 activity might have been induced by UVC. The remaining of FICZ in the UVB treated XEM2 cells was significantly lower than the untreated cells after 20 min incubation, but after 2 hours incubation, the metabolism of FICZ was not different from the control. Furthermore, the remaining amount of FICZ was higher after 2 hours than after 20 min. This inconclusive result indicated that more FICZ actually was being produced during the 2 hour incubation time than the amount being metabolized and could have been due to extra light exposure during the incubation. Significant difference between EROD and FICZ assay results may be due to the fact that EROD assay is not sensitive enough to detect the influence of UV light on CYP1A1 activity. UV light is known to be a strong inducer of DNA damage by gene deficient cells compared to the wild type cells that can repair most of their DNA damage. This is further validated with our result from both the DRAG assay, showing that cytotoxic effect for both UVB and UVC in NER deficient cells is significantly higher than in wild type cells (The IC<sub>50</sub> for UV5 being significantly lower than the IC<sub>50</sub> for AA8) and from the Hprt assay showing a significant difference in both survival and the mutant frequency between AA8 and UV5 cell lines treated with UVC light. These results show that UVB and UVC induce some kind of cytotoxic and mutagenic damage to DNA that are repaired by NER. It is known that both UVB and UVC lead to the formation of DNA adducts CPDs and (6-4) PP (Lippkeet *et al.*, 1981; Mitchelet *et al.*, 1989), which induce DNA lesions and can be partly repaired by NER pathway. These photo lesions are hypothesized to cause a cytotoxic effect in the cells causing transcriptional arrest, as well as inducing mutations during replication. However, it is also hypothesized that the repair system may be impaired by UV light. This might occur indirectly by interfering with signal transduction of certain genes (Daya-Grosjean *et al.*, 1995). Pyrimidine dimers cause the transition of C to T and CC to TT which are the most frequent mutations of p53 (Soehnge *et al.*, 1997). After UV exposure, cells activate p53 and stall the cell cycle for repair (Hermeking *et al.*, 1997). The repair system may be affected by altering the cell cycle to leave less time for DNA repair or by reducing the levels of enzymes that protect cells from UV damage (Daya-Grosjean *et al.*, 1995). Our study demonstrated the importance of a functional NER system that is repairing UV induced photolesions, for both cell survival and protection against mutations that might lead to cancer.

Treatment with the photosensitizer acetophenone is known to cause a shift in the production of photolesions toward mutagenic TT-CPD photolesion in cell cultures treated with UVB light (Biverstal *et al.*, 2008). Result from *hprt* assay also indicated that the high levels of TT-CPD photolesions, caused by treatment with the photosensitizer acetophenone, significantly increased mutation frequency in UVB treated cell cultures, even though an increased cytotoxicity with the photosensitizer acetophenone could not be proven. The doses of UVB used in the study might have been too low to see the cytotoxic effect of acetophenone. The study also showed that TT-CPD is more mutagenic than other CPD dimers. In the DRAG assay, IC<sub>50</sub> of UVB was about 20 times higher than UVC in the same cell type, indicating UVC to be more toxic than UVB. This is further validated through the results of *hprt* assay, which shows UVC light more mutagenic and cytotoxic than UVB light. Rating DNA adducts which can be recognized and repaired by NER system. The NER pathway in wild type cells will therefore repair the DNA damage caused by UV. However, NER deficient cells such as the UV5 cell line do not repair their DNA alterations as effectively as wild type cells. As a result, they are thought to be more sensitive to UV light induced cell toxicity. Due to this fact, the dose of UV light that inhibits half of the growth rate should be lower in NER

## CONCLUSION

The study concludes that UV light (UVB and UVC) causes the induction of metabolic activity of CYP1A1 by generating serious damage through photo products on DNA. Furthermore, our research showed that NER is an important DNA repair system for UV damage.

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