



Title	Assessment of ultraviolet toxicity depending on DNA-damage using cyclobutane pyrimidine dimer formation in ultraviolet irradiated cells [an abstract of dissertation and a summary of dissertation review]
Author(s)	Masuma, Runa
Citation	北海道大学. 博士(環境科学) 甲第11338号
Issue Date	2014-03-25
Doc URL	http://hdl.handle.net/2115/55363
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Type	theses (doctoral - abstract and summary of review)
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学 位 論 文 内 容 の 要 旨

環境起学専攻 博士（環境科学） 氏 名 Runa Masuma

学 位 論 文 題 名

Assessment of ultraviolet toxicity depending on DNA-damage using
cyclobutane pyrimidine dimer formation in ultraviolet irradiated cells
(UV 照射細胞におけるシクロブタンピリミジンダイマーを用いた DNA 損傷による
紫外線毒性の評価)

Ultraviolet (UV) radiation is a markedly prominent environmental toxic agent mainly emits from sunlight. Depending on etiological effects, UV radiation can be subdivided into UVA (315-400 nm), UVB (280-315 nm) and UVC (200-280 nm). UV radiation leads numerous complications like sunburn, erythema, epidermis hyperplasia, photo-aging, immunosuppression and photodermatoses. The harmful toxic effects mediated through UV radiation result from cumulative DNA damage. UV mediated DNA damage is mainly attributed directly by the formation of cyclobutane pyrimidine dimers (CPDs) between adjacent thymine or cytosine residues or to a lesser extent, formation of pyrimidine (6-4) pyrimidone photoproducts. Owing to its wavelengths, UVB and UVC are most proficient in induction of direct DNA damage through CPD formation. CPD formation is responsible for 70-80% DNA damage and found to be crucial for induction of relevant toxic effects of UV radiation. In absence of efficient DNA repair, CPD formation in the genome may leads to deleterious mutations which further actuate cytotoxicity and mutagenesis. Indefinitely, measurement of CPD formation considered as most sensitive assay for the determination of UV induced DNA damage. To date, how different wavelengths of UV radiation affect the mechanism of toxicity caused by UV radiation has not been investigated. The whole study is carried out for following three purposes; (1) to elucidate the different UV wavelengths (250 nm, 270 nm, 290 nm and 310 nm) mediated cytotoxicity, DNA damage and DNA repair on organisms by using PC12 cell system (2) to assess the protective potential of medicinal plant (*Tinospora cordifolia*) on UV-induced cytotoxicity and DNA damage in PC12 cell and (3) to find out the different cell sensitivity to UV radiation and molecular mechanism behind the UV sensitivity of CHO, NHEK and HUVEC cells compared to PC12 cell. To quantify cytotoxicity and DNA damage via CPDs formation, trypan blue exclusion assay and Enzyme linked immunosorbent assay (ELISA) were applied.

In first study, we evaluated cell viability for two purposes; one is to determine the cell killing ability of 4-different UV wavelengths at different exposure doses and the other is to evaluate the lethal doses (LD_{50}) of each tested UV wavelengths. On the other hand, quantification of CPD formation is implied to measure the DNA damage as well as DNA repairing ability of 4 wavelengths of UV radiation. Cell survival rate was markedly decreased 24 h after UV irradiation in a dose-dependent manner at all wavelengths (except at 310 nm). Cell viability increased with increasing wavelength in the following order: $250 < 270 < 290 < 310$ nm. UV radiation at 250 nm showed the highest cell killing ability, with a LD_{50} of 120 mJ/cm^2 . The LD_{50}

gradually increased with increase in wavelength. Among the 4 wavelengths tested, the highest LD₅₀ (6000 mJ/cm²) was obtained for 310 nm. CPD formation decreased substantially with increasing wavelength. Among the 4 wavelengths, the proportion of CPD formation was highest at 250 nm and lowest at 310 nm. On the basis of LD₅₀ values for each wavelength, PC12 cells irradiated with UV radiation of 290 nm showed maximum DNA repair ability, whereas those irradiated with the 310-nm radiation did not show any repair ability. Toxicity of UV radiation varied with wavelengths and exposure doses.

The safety of *Tinospora cordifolia* and its potential to protect against ultraviolet radiation-induced cytotoxicity and DNA damage in PC12 cells were investigated in second study. To evaluate the safety of *T. cordifolia*, cell viability, agarose gel electrophoresis, and TUNEL assay were carried out using PC12 cells treated with 0 to 100 µg/ml of methanol extract of *T. cordifolia*. To confirm the protective role against UV-induced damage, PC12 cells alone or in presence of 10 ng, 100 ng, or 1 µg/ml of *T. cordifolia* extract were exposed to 250, 270, and 290 nm of UV radiation, which corresponded to doses of 120, 150 and 300 mJ/cm², respectively. *T. cordifolia* extracts significantly increased cell viability at 1 ng, 10 ng, and 1 µg/ml concentrations in serum-deprived medium compared to control. *T. cordifolia* extracts did not show any cytotoxicity, and they inhibited apoptosis. Treatment with *T. cordifolia* extracts significantly increased the cell survival rate irradiated at 290 nm. In addition, *T. cordifolia* extracts significantly reduced CPD formation induced by UV irradiation at all wavelengths. In conclusion, *T. cordifolia* is not toxic and safe for cells. Our findings can support its application as phototherapy in the medical sector.

In third study, the 4 types of cells (PC12, CHO, NHEK and HUVEC) were used to assess the cell sensitivity to UV radiation. The cells were exposed to 250, 270, and 290 nm of UV radiation at 2-200 mJ/cm² exposure doses. As results, the cell survival rate of CHO and NHEK was significantly lowered at each tested wavelengths compared to PC12 cell. CPD formation results also revealed the similar trends. The cell sensitivity sequences according to cell viability and CPD formation were NHEK>> CHO≥ PC12. Different types of cell have distinctive cell sensitivity to UV radiation. Determination of prime causes of this discrepancy is great concern. To resolve this problem, molecular mechanism of UV radiated cells should be elucidated.

This study has been concluded as follows;

- (1) Till now, research has mainly focused on UVA and UVB radiation, and UVC remains poorly studied. Bio-analysis of toxicity of different wavelengths presented here is implicated to clarify cancer mechanisms and provide information that will be helpful in the fields of photobiology, dermatology, ophthalmology and cosmetology.
- (2) Identification and development of safe, non-toxic and effective radioprotective compounds are of enormous importance in mitigating the toxic effects of UV radiation. *T. cordifolia* has aptitude to alleviate the DNA damage that is mediated through toxic, lowered wavelengths of UV radiation and open a possibility of using *T. cordifolia* as photo-protective agent in medical sectors.
- (3) Identification of exact reasons of different cell sensitivity will provide new era in photobiology field and further helps in the research of oncology, biology and medical field.