A－4.5.1 Identification of factors for determining genetic susceptibility to ultraviolet

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Abstract We examined the dose rate-effect of UV-B irradiation on mutagenesis and related toxic effects. An action spectrum of UV-B and UV-A for mutation and cytotoxicity was also estimated. UV-B irradiation for a long duration at a low dose rate showed lower cytotoxicity than that for a short duration at a high dose rate, resulting that relative mutagenicity (number of mutations per input cells) might be elevated at the low dose rate. Our result suggests that a long exposure to weak ultraviolet is possibly more risky than a short exposure to strong ultraviolet.

Effect of UV-B irradiation on the embryogenesis of zebrafish and the mutagenesis in the embryo was investigated.

Key Words *HSV-tk* gene, mutagenesis, dose rate, malformation, action spectrum

Mutations induced in a mammalian cell line by ultraviolet (UV)-B and UV-A

Introduction

The ozone depletion is recognized to cause to elevate the irradiation of solar ultraviolet-B (UV-B, ultraviolet wavelength between 280-315 nm), which is filtered by the ozone layer. Increase in UV-B irradiation is a major concern about public health in recent twenty years, because it is suspected to increase in an incidence of skin cancer as consequences of mutation and imflammation caused by UV-B irradiation. Mutation potency of UV-B is required to be estimated for assessing the health risk for th increase of UV-B irradiatoin.

We have investigated ultraviolet-induced mutation using a rat fibroblast cell line stably infected with a retroviral vector carrying the neo and *HSV-tk* marker (LTK15/CREF) [1]. Our experiment demonstrated that monochromic UV-B induces weve length-specific mutation spectrum. In this study, we examined a dose rate effect of irradiation at 300 and 320 nm UV-B on the mutation and an action spectrum of UV-B and UV-A for mutation and cytotoxicity.

Materials and Method

LTK15/CREF cells were plated on a plastic dish, and irradiated after 16-20 h. A

monochromater (Biotronic, Vilber Loumat, France) equipped with an appropriate filter was used for the irradiation. Survival rate of the cells and mutation frequency (small mutations and large deletions) was determined as previously reported [2], but ganciclovir was used here for the selection of mutants instead of acyclovir.

In order to determine the does rate effect on mutation, the cells were irradiated to UV-B of 300 and 320 nm at the dose of 200 J/m^2 and 600 J/m^2, respectively, with different dose rates by changing a distance between a lump and the plate (9 - 25 cm). Mutations induced on HSV-tk gene were determined by PCR-direct sequencing.

Results

Structural comparison of the *HSV-tk* mutants detected after irradiation with 300 and 320 nm UV revealed (1) CC dimers and C oligomers as predominant targets at both wavelengths; (2) increased incidence of relatively large deletions at 300 nm (Fig. 1); and (3) greatly increased frequency of tandem double mutations at both wavelengths and clustered multiple mutations at 320 nm [2].

After the irradiation to 320 nm UV-B, survival of the cells was decreased, but number of the mutation was elevated with increase in the dose rate, indicating that relative mutatgenicity (mutations per input cells) was decreased depending on the increase in dose rate of irradiation. Interestingly, frequency of large deletions was rather low at the higher dose rate.

When 300 nm UV-B was irradiated, the relative mutatgenicity was also drastically elevated with increase in the dose rate. The relative mutatgenicity at the lowest dose rate was about 50-fold higher than that at the highest. Small mutation was major at low dose rate but not at high dose rate [3].

An action spectrum of UV-B and UV-A for mutation was very similar to that for cytotoxicity (Fig. 2). The relative mutagenicity and the relative lethality of cells was higher than absorption spectrum of DNA at the wave length below 320 nm. However, an acurate action spectrum has to be obtained using a more strong UV-B source.

Discussion

Effect of dose rate of UV-B irradiation on mutagenicity and cytotoxicity had not been well estimated. Generally, these effect of UV-B has been estimated under a condition of rather high dose rate. In this study, the mutagenicity and cytotoxicity were qualitatively and quantatively changed depending on the dose rate. UV-B irradiation for a long duration at a low dose rate showed lower cytotoxicity than that for a short duration at a high dose rate, resulting that relative mutagenicity might be elevated at the low dose rate. Our result suggests that a long exposure to weak ultraviolet is possibly more risky than a short exposure to strong ultraviolet. Since we are usually exposed to solar ultraviolet at a lower dose rate than the experimental comdition, further studies are required for health risk assessment of UV-B irradiation at a low dose rate.

Effect of UV-B irradiation on zebrafish embryo

Introduction

Biological effects of UV-B irradiation, especially mutagenesis, have not been quantatively examined on the level of animal body. Zebrafish is a good model vertebrate for investigating early development and mutagenesis. We are establishing genetically modified zebrafish (containing a gene of bacterial ribosomal protein, rpsL) to facilitate monitoring of mutagens in water. RpsL genes harbored on a shuttle vector were stably integrated on genomic DNA of zebrafish, and the integrated genes can be rescued efficiently into the host E. coli. In this study, zebrafish embryo of wild type and transgenic were exposed to UV-B, and phenotypic alteration of the larvae was observed and the mutations induced by UV-B were tried to be detected.

Materials and Methods

Embryos were exposed to 300 nm UV generated from a monochrometer 24 hrs after fertirization. Two or three days after exposure, the malformation of larvae was observed, and mutations induced by UV-B irradiation was determined.

Results and Discussion

As a result of 300 nm UV-B irradiation at the dose of 1,000 J/m^2, 80 % of larvae beared a disorder in the shape, etc a curved tail (Fig. 3). If 300 nm UV-B was irradiated to embryos of transgenic zebrafish, mutations were occured at the UV-specific sites (a pyrimidine dimer). Zebrafish may be applicable to determine solar UV-V for health risk assessment [4].

References

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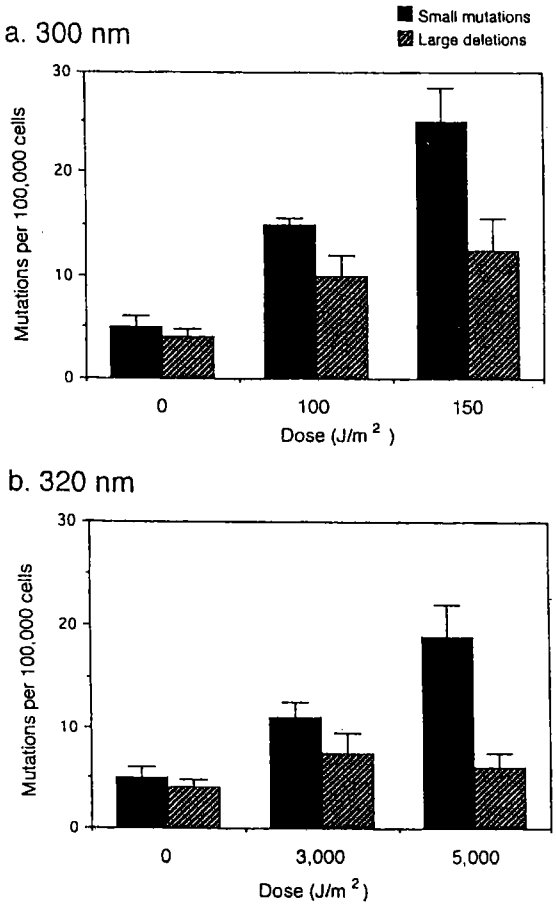
Fig. 1 (left) Proportion of small mutations (solid bars) and large deletions (striped bars) after irradiation by 300 nm (a) and 320 nm (b) UV.

Fig. 2 (right) Action spectrum for mutagenesis and cytotoxicity.



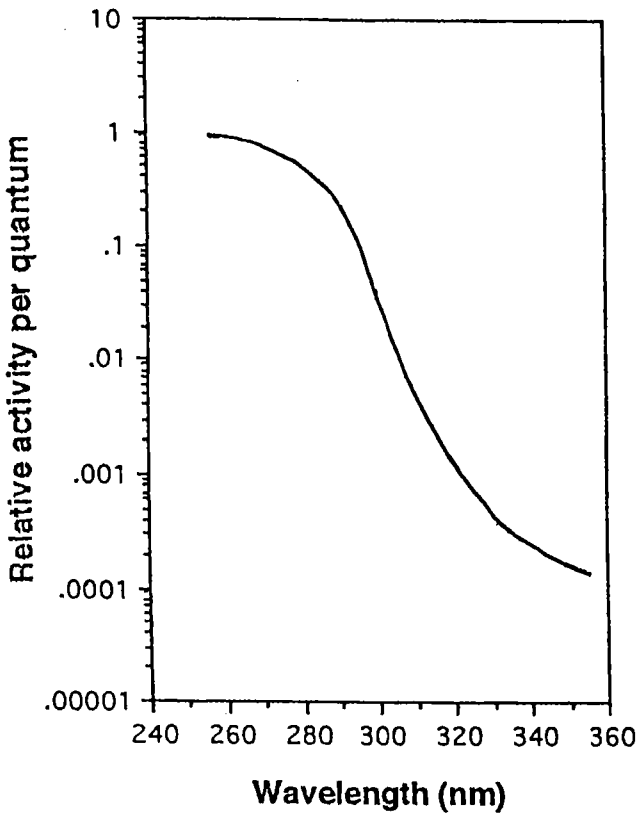


Fig. 3 Malformation of zebrafish larvae after 300 nm UV-B irradiation to the embryos.