Somatic Cell Mutation Induced by Sunlight in Drosophila

Tomoe Negishi 1, Shogo Takinami 1, Osamu Nikaido 2, Misato Mochizuki 1, and Megumi Toyoshima 1

There is ample epidemiological evidence showing that sunlight can cause skin cancer in the human. In experimental studies, simulated sunlight or UV lamps are used for demonstrating carcinogenesis and other biological effects. Little studies, however, have been performed using natural sunlight itself. In this work, we have examined the mutagenicity of natural sunlight in Drosophila. The Drosophila wing spot test is useful to detect somatic cell mutations. Third instar larvae in petri dishes were exposed to sunlight (ultraviolet region with <290 nm wavelength cut off by a plastic cover) in the yard of Okayama University campus (north latitude: 34° 39', east longitude: 133° 55'). The sunlight was mutagenic in Drosophila larvae and produced pyrimidine dimers in their DNA. In the observed mutagenicity, there was dependence on the exposure period and UV fluence. During the two-year monitoring, the highest induction of mutant spot observed was 1.98 total spots/wing on June 25, 1998, and the lowest was 0.64 on December 29, 1998, while non-exposure spontaneous spots were 0.29 and 0.32 on these days, respectively. Thus, solar radiation was mutagenic both in summer and in winter.


natural sunlight, somatic mutation, pyrimidine dimers, Drosophila

Epidemiological data show that solar radiation is carcino-genic in humans, and there are experimental results demonstrating that sunlight is indeed carcino-genic in rodents 1-3. An increased risk of skin cancer, possibly due to the increased UV radiation that was derived from the ozone layer-depletion, is a current issue in Australia 4,5.

There are numerous reports on experimental photocarcinogenesis 6. Although there are studies demonstrating mutagenicity and cytotoxicity of sunlight in cultured cells 7, bacteriophage 8,9, Bacillus subtilis spores 10 and yeast 11, no data are available on the sunlight mutagenicity in somatic cells. The Drosophila wing spot test is useful to detect the somatic cell mutation including gene mutation, chromosomal recombination, segmental chromosomal deletions, and nondisjunction 12. In this study, we have examined the mutagenic activity and lethality of sunlight using the Drosophila wing spot test. We have been able to detect somatic cell mutations in the Drosophila larvae that have been exposed to sunlight in the field of Okayama University. We have monitored the mutagenicity for 2 years, measuring the UV insolation alongside.

We also measured the amount of cyclobutane thymine dimers and (6-4) photoproducts in the DNA extracted from the sun-exposed Drosophila larvae; these DNA lesions are believed to be responsible for photocarcinogenesis 13,14.

MATERIALS AND METHODS

Materials

Drosophila melanogaster, y; Dp(1;3)sc14, y+ flr/TM1, Me ri sbdf males and y; mwh j v females were used in the wing spot test. These Drosophila strains were gifts of Dr. K. Fujikawa (Kinki University, Higashi-osaka) and Dr. H. Ryo (Osaka University, Suta).

DNase I (of bovine pancreas), snake venom phosphodiesterase and alkaline phosphatase (of calf intestine) were obtained from Boehringer Mannheim (Mannheim, Germany), RNase T1 (of Aspergillus oryzae) from Worthington Biochemicals (Freehold, NJ), RNase A (of bovine pancreas), spermine and spermidine from Sigma (St. Louis, MO), proteinase K (of Tritirachium album) from Merck (Darmstadt,
Germany), protamine sulfate (salmon roe) from Wako Pure Chemicals (Osaka, Japan), o-phenylenediamine dihydrochloride from Nacarai tesque (Kyoto, Japan), and biotin-F(ab')fragment goat anti-mouse IgG(H+L) and peroxidase-conjugated streptavidine from Zymed Laboratories, Inc. (South San Francisco, CA). For the measurement of photoproducts, we used 2 specific monoclonal antibodies; TDM-2 for cyclobutane thymine dimer and 64M-2 for (6-4)photoproducts in DNA 13).

Sunlight exposure and Drosophila wing spot test

The heterozygous larvae (mwh/flr and mwh/TM1) were obtained by mating of the virgin mwh-females with the flr/TM1-males16). The 3rd instar larvae were collected 72-96 h after oviposition and introduced at approximately equal numbers into tight-lidded plastic petri dishes (Toyo Roshi Kaisha Ltd., Tokyo; Ø 5 cm), which contained 1.5 ml of 0.25 M sucrose. Each dish had a hole covered with a nylon mesh for the exchange of air. The larvae in petri dishes were exposed to the sun, with the temperature held at 25±1°C, on a field in the campus of Okayama University (34° 39’N, 133° 55’E). Figure 1 is the diagram of the setting. The light of wavelength shorter than 290 nm was cut off by the plastic cover of the dish. During exposure, MED (minimal erythema dose) was recorded by an Erythema UV Intensity & Dose Meter (Solar Light Co., Philadelphia, PA), and UVA (ultraviolet A)- and UVB (ultraviolet B)-doses were measured by sensors (a 365 nm peak-sensor for UVA and a 310 nm peak-sensor for UVB) of UVX Radiometer (Uvp, Inc., Upland, CA). After the exposure, a part of the larvae were reared on a growth-diet (Formula 4-24, Carolina Biological Supply Co., Burlington, NC) to adult flies. The wings of the flies were screened under a microscope for mutant spots. The mutant spots were classified into small single, large single and twin spots according to Graf et al. 16. Statistical analysis was carried out as Frei and Würgler 17 and Kastenbaum and Bowman 18. The rest of the larvae were immediately frozen in liquid nitrogen and stored at -80°C until DNA extraction.

DNA extraction and measurement of photoproducts

The extraction of larval DNA was carried out as described previously 19. Frozen larvae were crushed into a fine powder and homogenized in a lysis buffer (100 mM Tris-HCl pH 8.0, 50 mM NaCl, 50 mM EDTA, 2% SDS, 0.15 mM spermine and 0.5 mM spermidine). The homogenate was treated with 0.2 mg/ml proteinase K for 1 h at 50°C. RNA was removed by digestion with RNase T1 (100 U/ml) and RNase A (500 µg/ml) at 37°C overnight, and then by dialysis against TE buffer overnight, using a nitrocellulose filter (pore size, 0.025 µm) (Nihon Millipore Ltd., Yonezawa, Japan).

Photoproducts were measured by an enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies as described previously 20. Denatured DNA samples were added to coat the wells of the protamine sulfate-pre-coated microtiter plate (Dynatech, Chantilly, VA) and dried. TDM-2 or 64M-2 was poured into the well coated with sample DNA and incubated at 37 °C for 90 min. After the incubation with biotinylated anti-mouse IgG and streptavidin conjugated with peroxidase, absorbance of colored solutions derived from o-phenylenediamine was measured at 490 nm using an immuno-reader (Immuno-Mini NJ-2300, Inter Med, Tokyo, Japan).

Figure 1. The apparatus for larvae exposure.
Figure 2. Mutagenicity, insolation and UV dose of natural sunlight in 1997.
A: Mutagenicities are shown as spots/wing, twin spot (■), large single spot (□□□), and small single spot (■). Survivals (●) are calculated according to the formula: the number of flies that emerged after exposure/the number of flies without exposure. B: Integrated UV dose are shown, UVA (●), UVB (○) and MED (▲). C: UV doses at every 30 min during exposure: UVA (●) and UVB (○). Symbols for weather: very clear (●), clear (▲), clear and later cloudy (●), clear and sometimes cloudy (▲), and cloudy (○).
Figure 3. Mutagenicity, insolation and UV dose of natural sunlight in 1998.
RESULTS AND DISCUSSION

We have monitored the mutagenicity caused by solar radiation in 1997 (Figure 2) and in 1998 (Figure 3). The mutagenicities increased depending on the exposure period and UV fluence within each day tested. The highest mutagenicity found was about 2 total spots/wing. We reported previously that a similar extent of mutagenesis was induced by UVA irradiation (320-400 nm, with black light) at about 200 kJ/m². On some clear days, when the sunlight was very strong (UVA 200 kJ/m²; UVB 100 kJ/m²), larvae were killed after a 4 hr exposure. The lowest mutagenicity, observed in December, was found to be still statistically positive.

As Figure 4 shows, both of the photoproducts in the larval DNA increased with the elongation of the exposure time up to 4 hr. On exposure for 6 hr, the photoproducts decreased. It is conceivable that the rate of photoproduct formation was reduced as the passage of day-time due to the decline of solar radiation, while the repair of photodamage stayed constant. The mutagenicities were in a stationary phase after the 6 hr-exposure.

During the two-year monitoring, sunlight in the summer was about 3-5 times more mutagenic than that in the winter. Although the mutagenicities increased depending on the UV fluences within a day examined, the correlation between the mutagenicities and UV fluences among 14 experiments for two years was not significant, as shown in Figure 5. It is suggested that the causes of mutagenic events are different among individual days. One possibility is that the light composition in sunlight may vary depending on the season and weather.

The UV dose declines in late afternoon, and yet the mutagenicity stayed constant (Figure 2, 3).

In this study, we demonstrated that sunlight causes somatic mutations in Drosophila. We consider that this Drosophila test may be useful as a model for higher organisms.

Figure 4. The amount of photoproducts in the larval DNA exposed to natural sunlight on October 21, 1997. 50 ng/well DNA was used to measure thymine dimer (●), and 1.2 μg/well DNA for (6-4) photoproduct (○).

Figure 5. The relationship between mutagenicities and UV fluences. The values of total spots/wing on 4 hr-exposure in each day are plotted against UV fluences of 4 hr-radiation; A: UVA, B: UVB and C: MED.
ACKNOWLEDGEMENTS

We thank Prof. Hikoya Hayatsu of the Faculty of Pharmaceutical Sciences, Okayama University, for his encouragement and many valuable suggestions for the present study, and his helpful advice in preparing this manuscript.

REFERENCES