A-4.2.1 Effect of ultraviolet ray increase on human health, Effect on immune depletion and the subsequent microorganism infection.

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Abstract The risk of solar UV ray-increase by ozone depletion to the human health was studied by looking at possible immune depression and the amplification of infectious diseases. In the case of immune depression, it was found that effects of UV-B ray was confirmed with delayed type hypersensitivity but no effect was seen in humoral immunity after vaccination. The effect of UV-B ray in interferon induction was dependent on the injection route of the stimulus. Experimental sunburn in mice increased plasma sialic acid which has been recognized as a member of immune family. UV-B ray on infectious diseases were also examined in vitro. In lytic infection of RNA and DNA viruses, pre-irradiation by UV-B ray as well as UV-C to cultured cells did not support but rather depressed the viral growth depending on the host cell death. In persistent infection, however, the viral genome DNA was amplified by not only UV-C but also by UV-B ray. Biological character of UV-B ray was different from that of UV-C especially in mutagenicity. Pathogenicity-lost mutant of pseudotuberculosis was generated by UV-C irradiation but not by UV-B ray. Herpes virus infection in mice is a positive evidence of the effect of UV ray on infectious disease in vivo. We confirmed that BALB/C mice lost their resistance to HSV as a result of UV-B ray pre-irradiation and those mice died from herpetic encephalitis within 2 weeks. In this project, the immunopathological background of the HSV disease in mice was studied.

Key ward: UV-B ray, Immune depression, Viral genome activation, Herpes simplex virus infection, Plasma sialic acid elevation.

Research objective

In this project, the effects of UV irradiation on immune function and the subsequent microorganism infection was studied. The theme was grouped into three categories.

Firstly, we focused on finding what immune functions were influenced by UV-B ray irradiation.

Secondly, the risk of increasing the infection by microorganism in vitro following UV-B irradiation was examined in virus-infected cultured cells and the effect of mutation on the pathogenicity of bacteria.

Thirdly, both the severity of the disease and immune depression in UV irradiated mice which were later infected with herpes simplex virus (HSV) was studied.

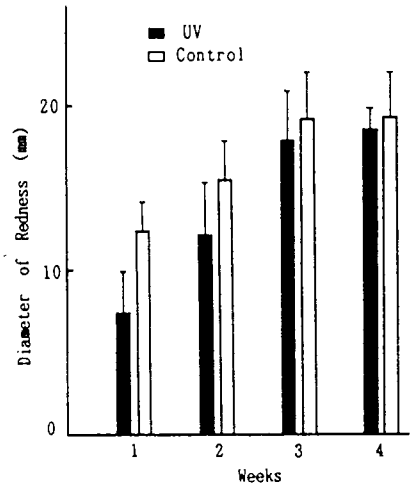
(1) Immunotoxicity of UV-B ray irradiation

Effect of UV pre-irradiation on antibody production after vaccination of formalin-killed influenza virus (IFV) to mice was studied. Dorsal (or abdominal) hair was shaved, cleaned by depilatory cream and exposed to a UV-B lamp (Toshiba FL20S.E, 40cm distance=0.065

mW/cm^2) for 60 min. In the course of sequential irradiation, at every week from 1 to 4, hand-made IFV vaccine (B/Kanagawa/1/63) of 280 HA was injected subcutaneously near the irradiation locus. After 3 or 4 weeks immunization, the serum HI titer were assayed and mean value of 4 mice was calculated. More than one week exposure to UV-B ray did not always depress the humoral immunity. Instead, short period irradiation such as just one day followed by inoculation at the next day showed slight lower production of HI antibody (<3.5 ±3.0) compared to the control (8.0±4.9). This might mean that the effect of UV irradiation on humoral immunity was transient and the mice would restore their antibody production capability within one week.

UV-B ray has been known to depress delayed type hypersensitivity (DTH). Many reports have described about contact hypersensitivity with agents such as picrylchloride. In this project, we tried to apply mycobacterium sensitization in guinea pig so as to induce DTH. The dorsal hair of guinea pig (350g) was shaved and exposed to UV-B ray (216 mJ/cm^2/day) for 3 days. At day 4, four guinea pigs ware challenged with lyophylized live BCG (0.1 mg) intracutaneously (ic.). On 1st, 2nd, 3rd and 4th weeks, purified tuberculin, PPD (0.2 μg) was inoculated ic. in different location from that of BCG. After 24 hr, diameters of redness were measured and the mean diameter was calculated. As shown in Fig. 1, in early sensitizing period (1-2 weeks), DTH of UV exposed guinea pig was significantly lower than that of the control. Thereafter, on the 3rd or 4th week, DTH had greatly progressed to a maximum to .rm65 equal the control. This means that irradiation of guinea pig leads to a delay in the onset of DTH because of T lymphocytes being unresponsive to BCG-sensitization.

Fig. 1. Effect of UV irradiation on DTH in guinea-pig sensitized with BCG



Interferon (IFN) is also important in the field of virology or immunology. Especially, IFN-γ is a key cytokine in immune network. UV irradiated mice (230 mJ/day for 3 days) which were sensitized with BCG were intravenously inoculated with 50 μg of purified tuberculin (PPD). Four hrs later, the circulating IFN was assayed with VSV on L-cells. IFN-γ titer in plasma from UV-B irradiated mice was not any different from control mice. On the other hand, when IFN-β was induced by poly I:C which was inoculated intracutaneously around UV-exposed area, the plasma IFN-β titer was half or less than that of control. Therefore, it seems like the production of IFN is dependent on route of injection.

Recently, it has become recognized that sialo-protein is a member of immune-families. In this study, experimental sunburn of mice by UV-B exposure showed an acute increase of plasma sialic acid. Since the increase showed a good correlation with UV dose, the level of plasma sialic acid may be applied to quantify the severity of sunburn and biological dose of UV-B in UV-irradiated animals.

(2) In vitro approach of UV risk on viral infection or mutagenicity of UV-B ray.

Viral infection following UV irradiation in cultured cells did not support the viral growth but the progeny virus titer was lowered depending on the host cell-death by UV-B or C ray.

UV risk on viral infection was also verified by viral genome amplification. In persistently infected cells with EB virus, the cell-associated episomal viral genome was not only amplified by UV-C but also by UV-B irradiation. When UV-B ray of 2 mJ/cm^2 was irradiated onto B95-8 cells persistently infected with EB virus, the viral genome was amplified by 5 to 10 fold compared to that of non-irradiated cells' when DNA was assayed as PCR product. However, in persistent papilloma virus infected cells, the episomal virus DNA was not amplified.

Mutagenicity of UV-C ray in some bacteria such as E.coli has been reported but the report on UV-B ray is scarce. In this study we examined mutagenicity of both UV rays with pseudotuberculosis of which pathogenicity was on plasmid but not chromosome. When UV-C ray of 17 mJ/cm^2 was exposed to the cells, the ratio of mutation was 1 to 10 of live bacterial cells. However, no mutant colony appeared on the agar-plate at any dose level and all the colonies died at 500 mJ/cm^2 of UV-B ray.

(3) General approach for the research of UV risk in infectious disease

Normal BALB/C mice are resistant to herpes simplex virus (HSV) infection. We found that UV-B ray pre-irradiation before HSV inoculation intracutaneously (ic.) had rendered normal BALB/C mice to be prone to HSV infection as the same zosteriform seen in nude mouse was demonstrated. When 134 mJ of UV-B ray was irradiated onto a shaved abdominal skin followed by HSV inoculation ic., 3 out of 5 mice formed zosteriform and at a high dose of 336 mJ, all mice died from herpetic encephalitis after forming pathological lesion.

To clarify immunological background, HSV specific immune response was studied by examining both antigen presentation (AP) and lymphocyte activation. Epidermal cells containing langerhans cells were prepared from ear pinnae of UV-B irradiated mice (360 mJ/cm^2) as accessory cells . The AP activity of the accessory cells in HSV specific immunity was determined from ^3H-thymidine incorporation into spleen T lymphocyte. The incorporated specific count was 40% of that of non-irradiated count meaning that there was a 60% inhibition of normal antigen presentation.

In the similarly irradiated and HSV-sensitized mice, the proliferation of HSV specific T lymphocyte from spleen or lymph node was determined by ^3H-thymidine incorporation. As shown in Table 1, 130 mJ of UV-B ray irradiation reduced the specific count to 68.2% (32% inhibition) in lymph node cells and 22.0% (80% inhibition) in spleen lymphocytes.

Table 1 Effect of UV-B irradiation* on HSV diseases and lymphocyte response.

UV-B	HSV	No of mice with zosteriform lesion	Incorporation of 3H-thymidine	
			Lymph node	Spleen
−	+	0/5	21,191±3,384**	16,566±2,358
+	+	3/5	14,467±3,005	3,638±339

* 135 mJ ** cpm SD

Results in past 3 years were summarized in Table 2.

Table 2 Effect of UV irradiation on immune function and infectious disease.

		UV-C	UV-B
In vitro	Mutagenicity to bacteria	+	−
	Viral growth (Lytic infection)	−	−
	Viral genome activation (persistent infection) (EB virus)	+	+
	(papilloma virus)	−	ND
In vivo	Humoral immunity	ND	depend on vaccination
	Cellular immunity (DTH)	ND	+
	Interferon production	ND	Local (+), Systemic (−)
	Infectious disease (HSV in mice)		
	Disease severity	ND	+
	Local immune function (AP activity)	ND	+
	Systemic immune function	ND	+
	Metabolism function ? (Plasma sialic acid)		+