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Effect of Ultraviolet Irradiation on the Development of *Trichinella spiralis*

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Abstract

The effects of short wave length ultraviolet-irradiation (UVC, 254 nm) on the development of *Trichinella spiralis* were studied. Non-irradiated larvae and larvae irradiated with different u.v.-doses were inoculated orally to 5-wk-old male ICR mice. Intestinal stage adult worms and muscle stage larvae were recovered at 7 days and 7 wks P.I., respectively. The recovery of both stages of nematodes was significantly reduced by the irradiation at 2 mJ/cm^2. The larvae irradiated by more u.v. dose greater than 5 mJ/cm^2 failed to develop to the adult stage and were expelled completely by 2 days of inoculation. Abortive infection was confirmed by the absence of muscle stage larvae in mice inoculated with 5 mJ/cm^2 u.v.-irradiated larvae. A few mucle larvae were, however, recovered from mice inoculated with 2 mJ/cm^2 u.v.-irradiated larvae. In conclusion, it is suggested that *T. spiralis* larvae are highly susceptible to u.v. at 254 nm of wavelength.

Key words: *Trichinella spiralis*; u.v.-irradiation; development.

Introduction

Outbreaks of trichinellosis were reported frequently in many countries of the world (Yamaguchi *et al.*, 1985; Ancelle *et al.*, 1985; Dissamarn and Indrakamhang, 1985; MacLean *et al.*, 1989). Therefore, prophylaxis against this zoonotic parasite is eagerly expected. In animal models of schistosomiasis mansoni, vaccination with u.v.-attenuated cercariae have been already developed for the analysis of the protective immunity (Ruppel *et al.*, 1990; Kamiya *et al.*, 1993). This notion should be also applicable to the possible development of vaccine for trichinellosis. Although Stowens (1942) and Stankiewicz and Jones (1983) reported the effect of u.v.-irradiation on *T. spiralis*, the appropriate attenuation-protocol has not been established as yet. To address this issue, effect of u.v.-irradiation on infectivity and growth of *Trichinella spiralis* was investigated.

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Materials and Methods

Parasite

Japanese isolate of *Trichinella spiralis* maintained in ICR mice (Yamaguchi *et al.*, 1975) was used throughout the experiments. Infective muscle-stage larvae of 7 wks P.I., were obtained from infected ICR mice by digestion in artificial gastric juice (0.8% pepsin and 0.8% HCl in 0.85% saline) for 3 hours at 37°C.

Animals

Male ICR mice were purchased from a commercial breeder (Funabashi Farm, Funabashi, Japan). All mice were used at 5 wks of age. All animals were fed food pellets (CE-2, CLEA) and water *ad libitum*. All animal experients in this paper followed the Guidelines for Animal experimentation of the Hirosaki University.

U.V.-irradiation of larvae and infection

U.V.-irradiation was carried out according to the method of Kamiya *et al.* (1993). Briefly, larval nematodes, approximately 200 larvae/ml were suspected in 2 mm-deep PBS (phosphate buffered saline, pH 7.2) in plastic petri dishes measuring 53

mm in diameter and irradiated by u.v. at 254 nm of wavelength with an u.v. lamp (Ultraviolet-Products Inc., California, USA) at a dose rate of 455 μW/cm^2 at room-temperature. The lamp was allowed to warm up for more than 20 min prior to use, to ensure a stable intensity of irradiation. The energy output was measured with a UVX Digital Radiatiometer (Ultraviolet Products). Irradiated larvae were inoculated to ICR mice by esophageal intubation within 30 min after the irradiation. The mean number±S.D. of infecting-irradiated larvae was counted exactly or calculated from 6 random aliquots. Non-irradiated larvae were also inoculated in parallel by the same manner.

Recovery of intestinal worms

Worms were recovered from the small intestine of mice at 7 days of infection. The small intestine was slit open and incubated 4 hours in 50 ml of PBS at 37°C. Then, the worms were counted under a dissection microscope.

Recovery of muscle-stage larvae

ICR mice inoculated with normal or u.v.-attenuated larvae were killed by ether inhalation 7 wks P.I. Then, muscle-stage larvae were collected by digestion of muscles with artificial gastric juice and then the number of larvae was recorded.

Statistic analysis

Statistic significance of the results was calculated using Student's *t*-test, with $P<0.05$ being taken as the minimal acceptable level of significance.

Results

Recovery of adult worms

According to information on the u.v.-irradiation of cercariae of *Schistosoma japonicum* (Moloney *et al.*, 1985), 1500, 1000, 500, 100, 20 mJ/cm^2 of irradiation were applied initially to attenuate the larvae of *T. spiralis*. With these doses, the larvae appeared to be very active *in vitro*. However, no worms were recovered from the intestine of mice 7 days P.I. Therefore, we examined the effect of the reduced doses of u.v.-irradiation (Table 1). After irradiation with more than 5 mJ/cm^2 the worms were not recovered at 7 days P.I. With 2 mJ/cm^2-irradiation recovery of worms was reduced significantly. Furthermore, 5 mJ/cm^2-irradiated larvae were not

Table 1 Recovery of adult worms from male ICR mice inoculated with u.v.-irradiated muscle larvae of *Trichinella spiralis* 7 days previously

Number of Experiment	Recovery (%) of intestinal worms after u.v. irradiation doses (mJ/cm^2)			
	0	2	5	10
I	25±9*	5±2†	0	0
II	33±6	19±4†	0	0
III	49±6	23±4‡	0	0

200 larvae were exactly counted and then inoculated to mice.
*Recovery = (number of worms recovered/no. of worms inoculated)×100, expressed by mean±S.D (n=5).
$^\dagger P<0.01$
$^\ddagger P<0.001$

Table 2 Kinetics of u.v.-irradiated larvae of *Trichinella spiralis* in intestine of male ICR mice

Groups	Number of larvae inoculated /mouse	Parasite recovery (%) after (hr)			
		12	24	48	72
Normal larvae	195±17	55±7*	58±4	54±13	56±10
5 mJ/cm^2-irradiated larvae	955±61	34±15	0.1±0.1	0	0

*Recovery = (number of worms recovered/no. of worms inoculated) × 100, expressed by mean±S.D (n=3).

Table 3 Number of muscle larvae recovered from male ICR mice infected with u.v.-irradiated larvae of *Trichinella spiralis* 7 wks previously

Number of Experiment	Number of larvae inoculated /mouse	Dose of u.v. irradiation (mJ/cm^2)			
		0	2	5	10
I	200	81±17	16±5‡	0	0
II	200	122±47	19±11†	0	0

* $\times 10^2$, mean±S.D (n=5).
† $P<0.01$
‡ $P<0.001$

retrieved as early as 48 hours P.I. (Table 2).

Recovery of muscle-stage larvae

Number of muscle-stage larvae deposited after inoculation with 2 mJ/cm^2-irradiated larvae was significantly lower than that with normal larvae. No muscle larvae were recovered from mice infected with 5 mJ/cm^2-irradiated larvae 7 wks P.I. (Table 3).

Discussion

The susceptibility to u.v.-exposure differs with the parasite species (Tromba, 1978; Molan and Al-Harmani, 1989; Karanis *et al.*, 1991). For instance, Ruppel *et al.* (1990) and Kamiya *et al.* (1993) have shown that u.v. dose of 15–18 mJ/cm^2 was appropriate to attenuate *S. mansoni* cercariae for the induction of protective immunity. On the other hand, 80 to 160 mJ/cm^2 of irradiation was required to attenuate *Trichomonas vaginalis* (Karanis *et al.*, 1991). Nevertheless, it is surprising that the development of *T. spiralis* larvae was significantly prevented by an irradiation dose as low as 2–5 mJ/cm^2. This remarkable sensitivity to *T. spiralis* to u.v. irradiation might be explained by its unique life cycle, since the parasite is never been naturally exposed to u.v. during their whole life cycle.

The mechanisms of u.v.-effect on parasite development have not been well understood. Since the larvae exposed to 5 mJ/cm^2 u.v.-irradiation were expelled in a relatively short time (Table 2), it is likely that the u.v. irradiation might cause an irreversible damage to parasite DNA (De Fabo and Noonan, 1990) or protein synthesis (Wales *et al.*, 1992). In the present study, 5 mJ/cm^2 u.v.-attenuated larvae were prevented for further development and rapidly expelled within 2 days with little pathological changes. Inhibition of protein synthesis was observed for u.v.- or gamma irradiated cercariae of *S. mansoni* and was suggested to be vital for induction of protective immunity (Wales *et al.*, 1992; H. Kamiya and J. D. McLaren, unpublished). Taken together, establishment of an animal model vaccinated with u.v. attenuated *T. spiralis* larvae will contribute to the development for prophylaxis and the prevention measures of trichinellosis.

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