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NOTE

Effect of ultraviolet radiation on the infectivity of *Taenia taeniaeformis* eggs

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Abstract

The effect of ultraviolet (UV) radiation on the infectivity of *Taenia taeniaeformis* eggs was observed. The eggs were exposed to various UV doses and orally inoculated to rats. The number of cysts and lesions decreased dose-dependently, and neither cyst nor lesion was observed from rats infected with eggs exposed to a total dose of 2,880 mJ/cm^2 or more. For evaluation of protective role of embryophore against UV radiation, the onchospheres with/without embryophore were exposed to UV radiation. Remarkably lower numbers of cysts and lesions were observed in rats inoculated with eggs which were exposed to a total dose of 30 mJ/cm^2 or more after removal of embryophore. These results suggested an importance of the protective function of the embryophore in the protection against UV radiation.

Key words: Eggs, *Taenia taeniaeformis*, Ultraviolet radiation

Recently the distribution of alveolar echinococcosis in Hokkaido, Japan, has been expanding. One of the most important routes of infection to human is thought to be through taking water or food contaminated with *Echinococcus* eggs. Therefore, it is an urgent and important requisition to establish an effective, convenient and economical method for sterilization of the eggs. One of the candidate methods for such purpose is an UV radiation which has been generally accepted for sterilization of infective organisms. Various effects of UV radiation on the infectivity, ability of growth, and pathogenicity of helminths have been studied[1)–10)]. Effective UV doses for inhibition of growth of various nematodes are 6–297 mJ/cm^2, but remarkable resistance to UV radiation was observed with *Echinococcus granulosus*[10)] (Table 1). Unfortunately, no further work to clarify the mechanism of the resistance to UV radiation and the minimal UV dose rate required to kill the taeniid eggs has been done. In this study, as an experimental model on UV effect for *Echinococcus*, preliminary analysis has been done on the effect of UV radiation on eggs with/without embryophore of *Taenia taeniaeformis*.

Gravid segments of *T. taeniaeformis* were collected from the feces of experimentally infected cats and were stored at 4°C in saline containing penicillin (100 IU/ml) and streptomycin (100 μg/ml) until used (within 1 month). The eggs were liberated from the segments by dissection. The UV lamp (254 nm, Iwasaki Electric Co. Tokyo, Japan) was allowed to warm up for 30 min prior to use and the UV dose rate was measured by a UVX radiometer (UVP, Inc., California, U. S. A.). The eggs were placed in 28 mm-diameter petri dishes added with saline at

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Table 1. Effective total dose of UV radiation on various helminths

Helminth	Total UV dose (mJ/cm^2)	Effect
Nematoda		
Trichostrongylus axei	30	Inhibition of larval growth to about (100%)[3]
Trichostrongylus colubriformis	30	Inhibition of larval growth to about (over 98.4%)[3]
Ostertagia ostertagi	30	Inhibition of larval growth to about (over 99.9%)[3]
Ostertagia circumcincta	297	Inhibition of larval growth to about (over 99.9%)[2]
Strongyloides papillosus	40	Inhibition of larval growth to about (100%)[7]
Stephanurus dentatus	6-12	Inhibition of larval growth to about (over 90%)[9]
Ascaris suum	30	Inhibition of larval growth to about (over 99.6%)[9]
Enterobius vermicularis	35	Inhibition of hatching (100%)[4]
Trichinella spiralis	138-228	Killing (100%)[8]
Trematoda		
Schistosoma mansoni	18	Inhibition of migration from skin to lung[5]
Schistosoma mansoni	24	Inhibition of cercarial growth to adult (over 99%)[6]
Schistosoma japonicum	24	Inhibition of cercarial growth to adult (over 99%)[6]
Cestoda		
Echinococcus granulosus	91,400	Inhibition of infection in intermediate host[10]
Moniezia expansa	2	Inhibition of infection in intermediate host[1]

1) Atmowisastro 1963 2) Bezubik et al. 1977 3) Herlich and Tromba 1980 4) Hollander et al. 1940 5) Kamiya 1993 6) Ruppel et al. 1990 7) Stankiewicz et al. 1970 8) Stowens 1942 9) Tromba 1978 10) Williams and Colli 1972

3 mm depth.

In experiment 1, the eggs with embryophore were exposed to UV (254 nm) radiation at a dose rate of 0.6 mJ/cm^2/sec with exposure time of 600–9,600 sec or 60 mJ/cm^2/sec with 6–192 sec. These eggs were stored at 4°C in saline containing penicillin (100 IU/ml) and streptomycin (100 μg/ml) for 3 days after irradiation and 5,000 eggs were orally inoculated to each of 12 male rats of 4-week-old (Slc : Wistar, Japan Slc , Hamamatsu, Japan). The rats were killed at 3 weeks post-infection. The livers were sliced at a thickness of 1–2 mm. The number of cysts and lesions in the livers was counted and clasified into 3 groups by diameter, small (< 1 mm, small tuberous lesion), medium (1–3 mm, medium tuberous lesion or cyst) and large (> 3 mm, large cyst).

The results are shown in Table 2. The number of cysts and lesions was reduced in rat groups inoculated with UV-irradiated eggs compared to the rat with non-irradiated eggs. In the group exposed at a dose rate of 0.6 mJ/cm^2/sec, 123, 48, and 1 cysts and lesions were observed in rats inoculated with the eggs exposed to a total dose of 360, 720 and 1,440 mJ/cm^2, respectively. With the increase of UV radiation, a decrease in the number of the cysts and lesions, especially of large size (normal size), was recognized. Neither cyst nor lesion was observed in the rats inoculated with eggs irradiated with 2,880 mJ/cm^2 or more. In the group exposed to a dose rate of 60 mJ/cm^2/sec, 110 and 16 cysts and lesions were also observed in the rats exposed to a total dose of 360 and 720 mJ/cm^2, respectively. As for the group exposed to a dose rate of 0.6 mJ/cm^2/sec, the reduction in the number of cysts and lesions, particularly of large size, was also observed with the increase of a total dose of UV radiation.

These results suggest that the infectivity of eggs or onchospheres and their ability of growth to strobilocercus are reduced depending on a total dose of UV radiation and also suggest that UV doses of over 2,880 mJ/cm^2 or more, which are considered to be quite high, are required to

Table 2. The number of cysts & lesions in the liver of rats inoculated with 5,000 *Taenia taeniaeformis* eggs irradiated at various doses of UV at 3 weeks post-infection

Dose rate & Radiation time		Total UV dose (mJ/cm^2)	No. & size of cyst & lesion[a]				Reduction rate[b] (%)
			Total	Small	Mediun	Large	
0.6mJ/cm^2/sec,	600sec	360	123	47	52	24	42.0
	1,200sec	720	48	29	17	2	77.4
	2,400sec	1,440	1	0	0	1	99.1
	4,800sec	2,880	0	0	0	0	100
	9,600sec	5,760	0	0	0	0	100
60mJ/cm^2/sec,	6sec	360	110	37	40	33	48.1
	12sec	720	16	7	5	4	92.5
	24sec	1,440	0	0	0	0	100
	48sec	2,880	0	0	0	0	100
	96sec	5,760	0	0	0	0	100
	192sec	11,520	0	0	0	0	100
No UV radiation		0	212	5	76	131	0

a: Small<1 mm in diameter, 1 mm ≦ Medium ≦ 3 mm, Large>3 mm

b: Reduction rate $= \left(1 - \frac{\text{Total no. of cyst \& lesion} - \text{No. of cyst \& lesion recovered at each dose of UV radiation}}{\text{Total no. of cyst \& lesion at no UV radiation}}\right) \times 100$

sterilize over 99% sterilization effect of *T. taeniaeformis* eggs.

In experiment 2, the role of embryophore in the resistance to UV radiation was evaluated by exposing the eggs with/without embryophore to UV radiation. In group 1, the embryophores of the onchospheres were removed by sodium hypochlorite solution before irradiation at a dose rate of 1.0 mJ/cm^2/sec with various radiation times, 30–4,860 sec and then the onchospheres were orally inoculated to each of 7 male rats of 4-week-old (3,000 onchospheres/head). In the group 2, the intact eggs were firstly UV-irradiated under the same condition as that of the group 1 before the removal of the embryophore. After the removal of the embryophore by sodium hypochlorite solution, these onchospheres were orally inoculated to each 5 male rats of 4-week-old (3,000 onchospheres/head). The onchospheres of both groups were kept for about 90 min after removal of embryophore and inoculated orally to rats. The rats in both groups were killed at 4 weeks post-infection and the number of cysts and lesions was counted in the same way as that in the experiment 1.

The results are shown in Table 3. Remarkably lower numbers of cysts and lesions were observed in the rats of group 1 at every total dose (30, 60, 180, 540, 1,620, 4,860 mJ/cm^2). In the group 2, 647, 130, 75 and 1 cysts and lesions were observed in the rats with total doses of 60, 180, 540 and 1,620 mJ/cm^2, respectively. As for experiment 2, the reduction in number of large cysts was also observed with the increase of a total dose of UV radiation.

The fact that UV radiation to the onchosphere without embryophore dramatically reduced the number of cysts and lesions suggests an importance of the protective function of the embryophore against UV radiation (group 1 in experiment 2). In the rats orally inoculated with eggs which retained embryophore during UV radiation (group 2 in experiment 2), the results were similar to those in rats in experiment 1. It is suggested that the effect of the treatment of onchosphere with sodium hypochlorite solution on the infectivity of *T. taeniaeformis* onchosphere could be neglected, because number of cysts and lesions in control inoculated with embryophore free eggs without UV treatment retained infectiv-

Table 3. The number of cysts and lesions in the liver of rats inoculated with 3,000 *Taenia taeniaeformis* eggs irradiated at various doses of UV at 4 weeks post-infection and protective role of embryophore against UV radiation

Dose rate & Radiation time	Total UV dose (mJ/cm^2)	No. & size of cyst & lesion[a]				Reduction rate[b] (%)
		Total	Small	Mediun	Large	
Group 1 (Radiation after removal of embryophore)						
1.0mJ/cm²/sec, 30sec	30	1	0	0	1	99.9
60sec	60	0	0	0	0	100
180sec	180	2	0	0	2	99.8
540sec	540	0	0	0	0	100
1,620sec	1,620	0	0	0	0	100
4,860sec	4,860	0	0	0	0	100
Group 2 (Radiation before removal of embryophore)						
1.0mJ/cm²/sec, 60sec	60	647	0	0	647	28.7
180sec	180	130	0	1	129	85.7
540sec	540	75	10	23	42	91.7
1,620sec	1,620	1	0	0	1	99.9
4,860sec	4,860	0	0	0	0	100
No UV exposure	0	908	1	17	890	0

a: Small$<$1 mm, 1 mm $\leqq$ Medium $\leqq$ 3 mm, Large$>$3 mm

b: Reduction rate $= \left(1 - \frac{\text{Total no. of cyst \& lesion–No. of cyst \& lesion recovered at each dose of UV radiation}}{\text{Total no. of cyst \& lesion at no UV radiation}}\right) \times 100$

ity and most of the cysts and lesions produced were large. With the increase of UV radiation, a decrease in the numbers of cysts and lesions, in particular of large cysts, was observed in the group 2.

The effective dose and wavelength of UV radiation on cestodes have not been determined. It was reported that the eggs of *Enterobius vermicularis* (nematode) were more sensitive at 228 and 280.5 nm than at 265 nm[4]. In our study, we used UV at only 254 nm, so that we must examine the effect of UV radiation with different wavelengths. On the other hand, the effective dose of UV radiation for *Moniezia expansa* (cestode) was low[1], but that for *T. taeniaeformis* in this experiment was quite high compared with that for other helminths[1)–10)]. Williams and Colli[10] reported the effect of a total dose of 91,400 mJ/cm^2 on eggs of *E. granulosus*. This suggested higher resistance of Taeniidae cestodes compared with other cestodes, trematodes and nematodes. As one of the reasons, we showed in experiment 2 that embryophore of *T. taeniaeformis* egg protect onchosphere, this is the first work showing the protective function of embryophore against UV radiation. It is suggested that effective UV radiation dose to *Echinococcus multilocularis* egg might be similar to that to *T. taeniaeformis* egg, because biological characteristics of *E. multilocularis* egg is similar to that of *T. taeniaeformis* egg, as both *E. multilocularis* and *T. taeniaeformis* belong to Taeniidae, whose onchosphere is covered with embryophore which consists of a thick structure of keratin blocks.

Although the embryophore plays a protective function against UV radiation in taeniid eggs, this study suggested that UV radiation is effective enough in sterilizing taeniid eggs such as *Echinococcus* eggs.

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