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Protection of Mice against *Echinococcus granulosus* by Previous Inoculation with Protoscoleces Exposed to Ultraviolet Irradiation

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Abstract

The effects of ultraviolet (UV) irradiation on motility, infectivity, and vaccine potential of protoscoleces of *Echinococcus granulosus* were studied. An increase in the exposure of the protoscoleces, *in vitro*, to UV-irradiation results in an increase in mortality. Exposure of protoscoleces to UV-irradiation for 75 minutes was necessary to immobilize immediately all the larvae, whereas exposure for 45 minutes rendered them noninfective. Intraperitoneal inoculation of mice with protoscoleces exposed to UV-irradiation for 70 or 75 minutes conforred considerable protection, whereas all those inoculated with larvae which had been exposed to UV-irradiation for 45, 50 or 60 minutes showed a 100% resistance to challenge infection.

Key words: *Echinococcus granulosus*, Protoscoleces, UV-Irradiation, Resistance, Mice.

Introduction

Echinococcosis or hydatid disease is a cyclozoonotic infection caused by larvae of the two closely related species of cestode, *Echinococcus granulosus* and *E. multilocularis*. The disease is an important public health and economic problem so far as it affects both man and his livestock and is found in varying degrees on every continent (Abdusslam *et al.*, 1968).

Hydatid infections constitute a major health problem in Iraq and their seriousness has been recognized by Babero *et al.* (1963), Hassoun and Al-Salihi (1973), Niazi (1974) and Mahmoud (1980). Over 500 cases of human illness are recognized yearly with a wide variety of clinical manifestations (Babero *et al.*, 1963, Niazi, 1974; Molan *et al.*, 1987).

Although vaccines consisting of irradiated helmith parasites have been reported against nematodes (Jarrett *et al.*, 1960; Mulligan *et al.* 1961; Miller, 1965; Tromba, 1978; Herlich and Tromba, 1982; Menon and Bhopale, 1985), and trematodes (Villella *et al.*, 1961; Radke and Sadun 1963; Ghandour and Webbe, 1975; Hsu *et al.*, 1982), only infrequent attempts to immunize against cestodes with ionizing radiation have been reported (Urqhart, 1961).

The present investigation was undertaken to study the effects of ultraviolet irradiation on the infectivity and survival of the protoscoleces of *Echinococcus granulosus* and to determine the extent to which the resulting attenuated larvae could produce protective immunity in mice.

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Materials and Methods

Experimental animals

Healthy male 4-week-old Balb/c mice, were used throughout this investigation. They were kept in metal cages and fed with pellet food and water *ad libitum*.

Parasite and infection

Hydatid fluid was withdrawn from fertile cysts in the viscera of freshly slaughtered sheep and the cyst was cut open and the brood capsules removed and rinsed in Hanks solution and then treated with 0.5% pepsin (PH. 2.0) and incubated (15 ~ 30 minutes) until protoscoleces released (Smyth, 1985). The protoscoleces were counted by the use of Neubauer

slide. The viability of the protoscoleces was determined by movement, flikering of the flame cells, and impermeability to 0.1% aqeous eosine stain. The protoscoleces were injected intraperitoneally into recipent animals. The mice were asphyxiated by ether and their abdomens and chests opened. The exposed cysts were gently freed into a petri dishes and then counted and measured.

Ultraviolet irradiation of protoscoleces.

The source of irradiation was ultraviolet lamp of 2537 Å. A suspension of 50,000 larvae in 5 ml Hanks solution in a glass petri dishes with 5 mm depth of larvae suspension, was exposed (at room temperature 22 ± 2°C) to UV-irradiation at a distance of 5 cm from the source. This distance was kept constant for the exposure in all the experiments. The UV-tube was switched on for 10 minutes before the exposure of larvae in order to obtain even emission of radiation.

Experimental design.

Experiment 1. To determin the effect of UV-irradiation on mortality, *in vitro*.

Protoscoleces were exposed to UV-irradiation for 10 to 75 minutes (Table 1). Samples of between 100 and 200 protoscoleces were removed from the experimental dishes, and examined under the microscop 1 hr, 5 hr, 10 hr, 24 hr, 2, 3, 4, 5, 10, and 15 days after exposure to irradiation. The number of protoscoleces killed by irradiation is expressed as a percentage of the total number in the sample (Table 1).

Experiment 2. Development of UV-irradiated protoscoleces in mice.

Twelve baches of larvae were dispensed in petri dishes, each of which contained 30,000 larvae per 3 ml Hanks solution and exposed to UV-irradiation for 2, 4, 7, 10, 20, 25, 30, 40, 45, 50, and 60 minutes respectively. Twelve groups, each of 4 mice, were injected intraperitoneally with a dose of 4000 UV-irradiated larvae of different time exposure. Four mice in another group were infected similarly with 4000 UV-irradiated larvae of different time exposure. Four mice in another group were infected similarly with 4000 normal larvae to serve as control. All mice were killed 90 days later for the recovery of secondary hydatid cysts from the viscera.

Experiment 3. Evaluation of UV-irradiated *E. granulosus* larval vaccine.

Five groups each of 4 mice, were inoculated intraperitoneally with a dose of 4000 larvae which had been exposed to UV-irradiation for 45, 50, 60, 70, or 75 minutes respectively. Thirty days later mice in all five groups, together with 4 previously uninfected mice, were challenged per os, with 2000 normal larvae. All the animals were killed and dissected 60 days after challenge.

Table 1 *In vitro* survival of protoscoleces of *E. granulosus* exposed to UV-irradiation

Exposure time (min)	Percentage of survived larvae									
	Time after irradiation									
	1 hr	5 hr	10 hr	24 hr	2 days	3	4	5	10	15 days
0	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	55	0
20	100	100	100	100	80	45	19	0		
30	100	100	93	70	25	0				
40	94	68	29	0						
50	85	43	11	0						
60	53	17	3	0						
70	21	0								
75	0									

Standard deviations of the mean were calculated and the statistical significance of the results analyzed by Student "t" test. $P < 0.05$ is considered statistically significant.

Results

Experiment 1.

Table 1 presents the data concerning the percentage of larvae which survived *in vitro* after exposure for different times (from 10 to 75 minutes) to UV-irradiation. All larvae were killed one hour after exposure to 75 min irradiation, and only 21% of larvae survived one hour after 70 min irradiation. All larvae were killed one day after exposure to 40, 50, or 60 mins. A small proportion of larvae (19%) survived 4 days after exposure to 40 mins irradiation, and more than 50% of them survived 10 days after exposure to 10 mins irradiation. It is very interesting to note that all normal larvae (non-irradiated) were killed after 30 days.

Experiment 2.

It can be seen from Table 2 that as the time of exposure of the protoscoleces to UV-irradiation was prolonged from 2 to 40 minutes, the number of secondary hydatid cysts developed was correspondingly reduced from 12.25 ± 7.5 to 0.42 ± 0.49. The mean number of cysts of the control group was found to be 36.25 ± 16.0 (Table 2). It is interesting to mention that no specific site for cyst development was favoured more than any other, and developing cysts were found scattered throughout the peritoneal cavity, either free or adhering to various sites including liver, spleen and some cysts were also frequently attached to the kidneys. It was found that mice inoculated with protoscoleces exposed to UV-irradiation for 10, 20, 30, and 40 mins harbour significatly fewer ($P < 0.01 \sim 0.001$) cysts than their counterparts infected with non-irradiated larvae. Ex-

Table 2 Mean number and size of secondary hydatid cysts recovered from mice, 4 in each group, after intraperitoneal inoculation with 4000 UV-irradiated protoscoleces at different time exposure. All mice were necropsied 90 days after infection

Exposure time (min)	Mean no. of cysts ±SD	Mean size (mm) ±SD (range)	Proportion of animals infected	% inoculated larvae developed into cysts
0	36.25±16.0	0.83±1.1 (0.3-3.7)	4/4	0.91
2	12.25± 7.5	1.08±0.75 (0.5-1.5)	4/4	0.31
4	6.0 ± 7.8	1.08±0.98 (0.5-4.0)	4/4	0.15
7	1.67± 0.57	1.00±0.35 (0.5-1.5)	4/4	0.04
10	2.33± 4.35	0.97±0.37 (0.5-1.5)	4/4	0.058
15	5.75± 3.25	0.84±0.44 (0.5-2.0)	4/4	0.14
20	1.50± 1.00	1.16±0.25 (1.0-1.5)	4/4	0.021
25	1.33± 1.00	1.12±0.25 (0.5-1.5)	3/4	0.03
30	1.25± 0.43	0.87±0.52 (0.5-2.0)	3/4	0.03
40	0.42± 0.49	0.83±0.28 (0.5-1.0)	2/4	0.006
45	0	—	0/4	0
50	0	—	0/4	0
60	0	—	0/4	0

Table 3 Secondary hydatid cysts recovery from mice inoculated with 4000 UV-irradiated larvae followed by 2000 normal larval challenge infection

Exposure time (min)	Mean no. of cysts ± SD	Mean size of cysts (mm) ± SD	Proporation of animals infeted	% inoculated protoscoleces developed into cysts
0*	10.25 ± 4.27	0.68 ± 0.25	4/4	0.51
45	0	—	0/4	—
50	0	—	0/4	—
60	0	—	0/4	—
70	3.25 ± 1.26†	0.47 ± 0.16	3/4	0.16
75	8.0 ± 2.55‡	0.55 ± 0.17	4/4	0.40

* Control (non-immunized)
† $P < 0.001$ vs. control by Students t test.
‡ Not significant vs. control.

posure of protoscoleces to UV-irradiation for 45 mins and longer destroys the infectivety totally as no cysts were established in mice inoculated with 4000 larvae which had been irradiated for 45 mins or longer (Table 2).

Experiment 3.

The results presented in Table 3 show that intraperitoneal inoculation of 4000 larvae exposed to UV- irradiation for 70 or 75 mins conferred considerable protection against challenge infection with 2000 normal larvae, whereas 4000 irradiated (exposed for 45, 50, and 60 mins) larvae gave complete protection against challenge infection as evidenced by absence of cysts at autopsy when examined after killing on day 60 post challenge infection. Control mice, infected with 2000 normal protoscoleces, developed infection in 4/4 instances (Table 3) and the mean number of cysts was 10.25 ± 4.27.

Discussion

The progressively damaging effects of increasing time of exposure to UV-irradiation on the protoscoleces of *Echinococcus granulosus*, which include increasing mortality *in vitro*, and decreasing survival, infectivity and development to cystic stage *in vivo*, are similar to those reported by many authors on other parasite species (Standen and Fuller, 1959; Keeling, 1960; Katiyar *et al.*, 1968; Stankiewiez *et al.*, 1970; Gandour and Webbe, 1975; Prevati and Chopra, 1975; Benzubic and Wedrychowicz, 1976; Molan, 1983; Menon and Bhopale, 1985).

The present results revealed that the larvae exposed for 45, 50 or 60 mins to UV-irradiation were sluggish but conferred 100% protection against the challenge infection of normal non-irradiated larvae. Similarly Katiyar *et al.* (1968) stated that subcutaneous injection of 20000 larvae of *Nippostrongylus brasiliensis* which had been exposed to UV-irradiation, gave complete protection against a challenge dose of 10000 normal larvae. Benzubik *et al.* (1977) in their studies with *Ostertagia circumcinta*, suggested that both normal larvae and larvae exposed to 30 mins to UV-rays were able to stimulate the defence mechanism of sheep but the irradiated larvae eliminated the pathogenicity of the worm to a greater degree and a few larvae completed the full life-cycle and stimulated the production of specific antibody by the host. Recently, Menon and Bhopale (1985) mentioned that one oral vaccination of hamsters with 100 infective larvae exposed to UV-irradiation for different time intervals induced the development of resistance. The more efficient induction of resistance by irradiated larvae, described here (larvae exposed for 45, 50, or 60 mins to UV-rays) and by Jarrett *et al.* (1960), Tromba (1978) and Menon and Bhopale (1985) may be due to an initial increase in the

leakage of antigenic metabolites by the damaged parasites. Increasing exposure to irradiation (70 or 75 mins), however, may cause so much damage as gradually reduce metabolic activity until death, when no metabolites are released and less or no immunity is induced. Similarly, Mulligan (1975) found that 20 or 40 Krad X-irradiated larvae induced immunity to *Dictyocaulus viviparus* in calves but no immunity resulted from the administration of dead larvae which had been treated with radiation dose of 69 Krad.

Comparison of the effects of UV-irradiation on protoscoleces of *E. granulosus* with that X-rays (Movsesijan *et al.*, (1968) suggests that UV-irradiation is more damaging than X-rays. As Movseijan *et al.* (1968) demonstrated that the protoscoleces survive doses of 10, 20, 25, and 30 Kr of X-rays, on the other hand Ohnishi (1986) who studied the effect of X-ray irradiation of the proliferative ability of the germinal layer cells of larval *E. multilocularis*, stated that a dose of 55 Kr was needed to abolish the infectivity totally. In our study exposure of protoscoleces to UV-irradiation for 45 minutes destroys the infectivity completely (Table 2).

In conclusion, this study provide a simple, rapid, and inexpensive procedure as compared to gamma or X-irradiation. This should allow a method for the induction of protective immunity to experimental hydatidosis which can be performed in most laboratories using UV-lumps without access to gamma or X-irradiation equipments.

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